Forget foraging - Designer proteins are becoming a reality

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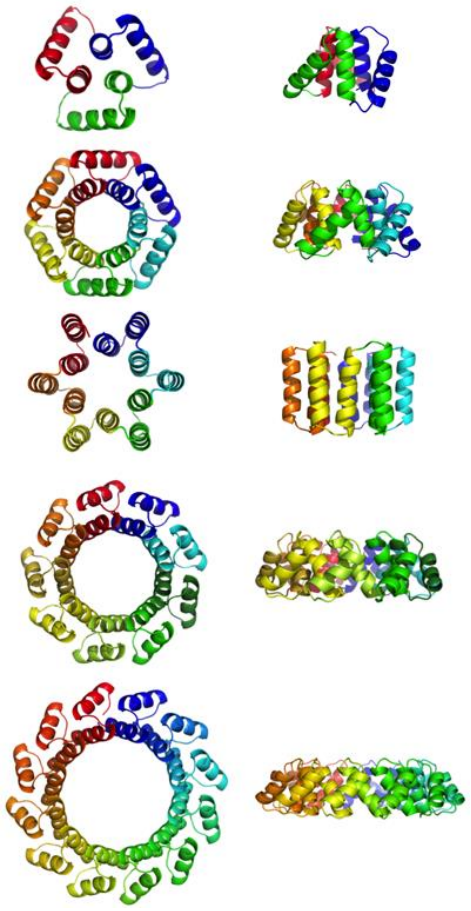

Top (left) and side (right) views of the five structures of designed monomeric tandem repeat proteins chosen for purification. From top to bottom the proteins are of the 3- repeat, 6- repeat (two structures), 9-repeat and 12- repeat classes. The proteins are colored from blue to red from amino to carboxy terminus.

Image provided by Dr. Phil Bradley.

Protein modeling and design is a field that has made significant advances in recent years, advances that have enable researchers to pursue lofty and increasingly translational goals. Bioengineers aim to understand the range of protein structure "space" that can be designed, folded into a stable structure, purified and put to a good use. Tandem repeat proteins are especially attractive as a design target for both aesthetic and bioengineering purposes, as they could potentially form stable vehicles or platforms for therapeutics. Tandem repeat proteins, which contain repeated segments of structural motifs, that could self-associate to form a closed structure are ideal for therapeutics as well as purification because they avoid the problem of oligomerization (the stable association of more than one protein in higher-order complexes).

Dr. Phil Bradley (Public Health Sciences Division) wanted to put his structural know-how to the test and design and make tandem repeat proteins that "close" themselves unlike any of those found in the natural world to date. The product of this vision and close collaboration with structural biologist Dr. Barry Stoddard (Basic Sciences Division) was recently published in *Nature*.

Dr. Bradley wrote an algorithm to design tandem repeat proteins that could be used within the Rosetta molecular modeling package developed in David Baker's Lab (UW Biochemistry). Rosetta designs proteins through an iterative process and the algorithm Dr. Bradley wrote contains extra features which ensure the symmetry of the protein "backbone" as well as the regularity of the structure and spacing of each segment in these tandem repeat proteins. Following structure prediction, the authors chose to focus on structures with left-handed bundles, as this architecture appears totally absent from the known protein structure database (http://www.rcsb.org/pdb/home/home.do). They selected proteins with several different numbers of repeats, ranging from 3- to 12- repeated segments.

Research technicians Lindsey Doyle, Jazmine Hallinan and Jill Bolduc (Stoddard Lab, Basic Sciences Division) purified and determined the structure of proteins representing four different classes of repeat proteins (3-repeat, 6-repeat, 9-repeat, and 12-repeat structures) by X-ray crystallography (see Figure). The donut-shaped or toroid structures were solved to high resolution (2-3 Angstroms) and only varied slightly (0.6-1.1 Angstrom) from the predicted structures in root mean square (RMS) deviation of alpha carbon atoms. The fact that it was possible to purify and characterize the structure of tandem repeat proteins with different numbers of repeats suggests that the central 'pore' of the protein could be manipulated by altering the number of repeats. Researchers in the Stoddard Lab further characterized the proteins by size-exclusion chromatography and found that the 3- and 6-repeat proteins formed stable dimers in solution while the 9- and 12- repeat proteins formed monomers. The biochemical behavior of the dimers and monomers did not vary significantly over different protein or salt concentrations, suggesting their potential utility as stable therapeutic agents.

To test the flexibility of the system, the scientists wondered if they could divide the 9-repeat structure into 3 equivalent pieces that would self-assemble and form the same size ring. In doing this, the authors discovered that the smaller subunits formed a completely different pattern when packed together in the crystal lattice. Rather than forming a ring with 3 subunits, the proteins instead formed a larger ring with four subunits. Amazingly, this structure formed an interlocking set of rings resembling a chain. This structure, as well as layers of 9- and 12- repeat proteins that form tubes, have the potential to be developed for novel drug delivery platforms or nanomaterials. In addition to

this, by changing the amino acids that line the central pore, it may be possible to build proteins that aid in catalysis or drug presentation. "We are optimistic that these highly stable designed proteins will be useful as scaffolds for engineering new functional domains. For example, we are investigating whether we can insert multiple binding domains into these structures and create signaling proteins with high avidity for their receptors," said Dr. Phil Bradley. The future of bioengineering is bright indeed.

Doyle L, Hallinan J, Bolduc J, Parmeggiani F, Baker D, Stoddard BL, Bradley P. 2015. "Rational design of alpha-helical tandem repeat proteins with closed architectures." *Nature*. 528:585-8.

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