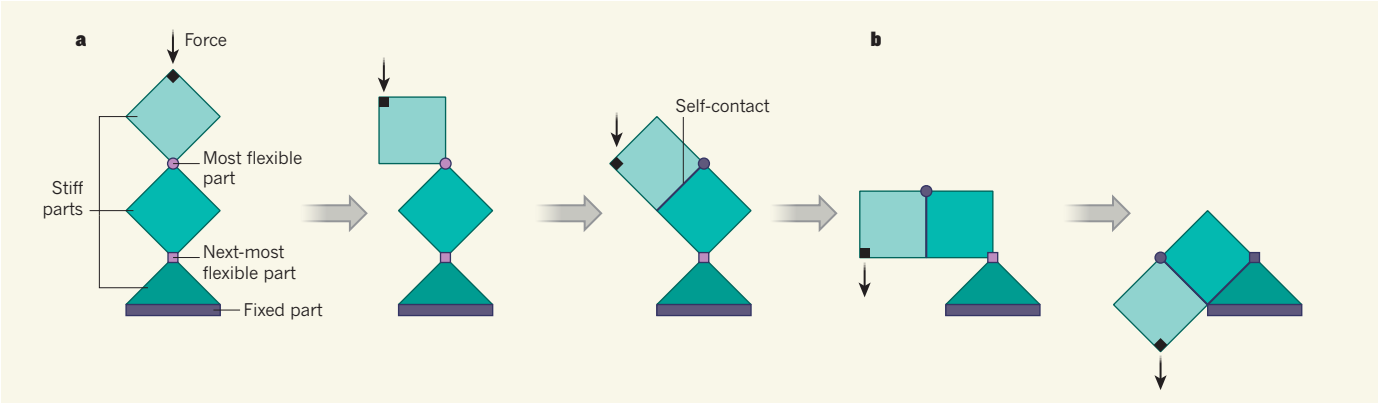


Figure 1 | Self-guided, multi-step motions of a mechanical structure. Coulais *et al.*[1] demonstrate an approach for designing materials that follow a multi-step pathway consisting of a series of shape changes, without needing external control. The materials are composed of building blocks (unit cells), and a subset of a unit cell is shown here. **a**, The subset consists of stiff parts that are connected by flexible parts, and a part that is fixed. The flexible parts have different degrees of flexibility. The authors apply an external force to the structure (at the point marked by the black square), which causes the most flexible part to bend. This bending moves one of the stiff parts until it connects with another stiff part — a process known as self-contact. The subset reconfigures such that the most flexible part becomes inactive and the connected parts act as a single stiff part. The first step of the multi-step pathway is complete. **b**, The next-most flexible part then bends until another self-contact occurs, which prevents further motion. The desired configuration is reached, and the second step of the pathway is concluded.

connected by flexible parts (see Fig. 1a of the paper[1]). In the first step of the multi-step pathway, each unit cell rotated to produce a structure of differently sized squares. In the second step, each of these squares rotated to fill an assigned space.

Coulais and colleagues illustrated the concepts associated with their approach using only a few specific examples, but their work provides guidelines that will enable the design of other shape-changing metamaterials. The authors' unit cells can be configured in many different ways, resulting in different motions. For example, two structures that have nearly identical topologies can have contrasting pathways (see Fig. 1a,b of the paper[1]). Even more configurations are made possible by producing different arrays of the unit cells, or by using the principles discussed in the paper to make other unit cells and arrays.

The approach does have some limitations. For instance, the flexible parts are relatively small and undergo 90° of rotation, and few materials can withstand such large strains without breaking. Even materials that do not break might still become permanently deformed, making the process irreversible — and therefore unsuitable for many applications. The authors used 10-millimetre-thick silicon rubber for the flexible parts in their experiments. Although the use of this material is valuable for verifying and demonstrating the approach, issues will probably arise when smaller parts are required, because the material choice will be limited.

Coulais and colleagues' results open up many avenues for future research. A natural next step would be to see what other multi-step motions can be realized using the approach. Researchers could also study how the designs vibrate when they are shaken, to see whether they still undergo the intended movements, or whether they follow a different pathway.

In addition, it would be useful to explore whether switch-type mechanisms[8,9] can be used to snap the structures into desired configurations, replacing the self-contact function and perhaps eliminating the need for an external force to keep the structures in key intermediate positions. Finally, researchers could investigate how the principles described in the current work could be used to produce sophisticated motions beyond multi-step pathways, and how the approach might be applied to the micrometre and nanometre scales. ■

Larry L. Howell *is in the Department of Mechanical Engineering, Brigham Young University, Provo, Utah 84602, USA.*
e-mail: lhowell@byu.edu

1. Coulais, C., Sabbadini, A., Vink, F. & van Hecke, M. *Nature* **561**, 512–515 (2018).
2. Overvelde, J. T. B., Weaver, J. C., Hoberman, C. & Bertoldi, K. *Nature* **541**, 347–352 (2017).
3. Reis, P. M., Jaeger, H. M. & van Hecke, M. *Extreme Mech. Lett.* **5**, 25–29 (2015).
4. Haghpanah, B., Salari-Sharif, L., Pourrajab, P., Hopkins, J. & Valdevit, L. *Adv. Mater.* **28**, 7915–7920 (2016).
5. Howell, L. L., Magleby, S. P. & Olsen, B. M. *Handbook of Compliant Mechanisms* (Wiley, 2013).
6. Hopkins, J. Shaw, L., Dotson, M., Chizari, S. & Song, Y. *Bull. Am. Phys. Soc.* abstr. F17.00004 (2018).
7. Mankame, N. D. & Ananthasuresh, G. K. *Comput. Struct.* **82**, 1267–1290 (2004).
8. Silverberg, J. L. *et al. Nature Mater.* **14**, 389–393 (2015).
9. Waitukaitis, S., Menaut, R., Chen, B. G. & van Hecke, M. *Phys. Rev. Lett.* **114**, 055503 (2015).

COMPUTATIONAL BIOCHEMISTRY

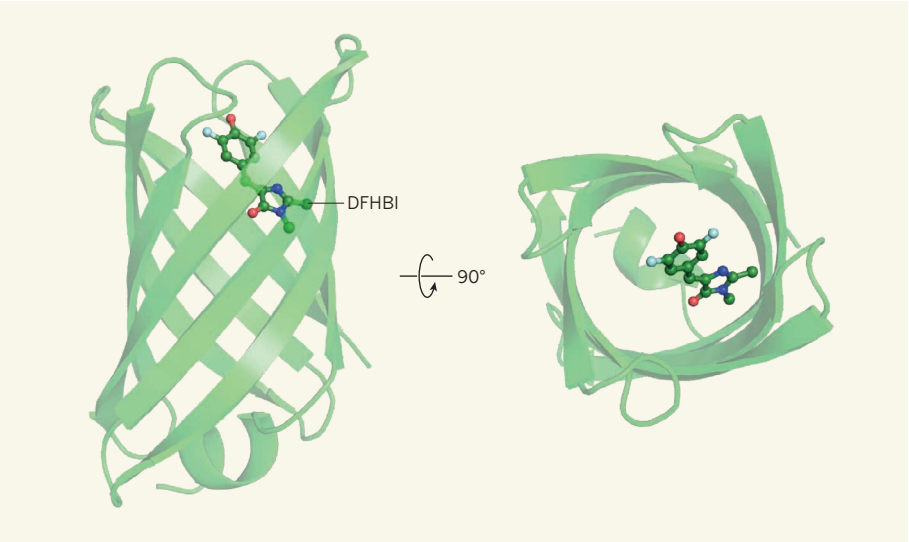
Designer proteins activate fluorescence

A computational method has been devised that allows a structural motif found in proteins, known as a β-barrel, to be designed to bind specifically to any small molecule, opening the door to biotechnological applications. SEE ARTICLE P.485

ROBERTO A. CHICA

Proteins are the molecular machines of life: they carry out the complex molecular processes required by cells with unrivalled accuracy and efficiency. Many of these processes depend on proteins having the ability to bind specifically to a given small molecule. If we could make proteins from scratch to bind any desired target molecule, it would open the door to a wide range of biotechnological applications that are not currently possible using natural proteins. On page 485, Dou *et al.*[1] describe a computational method for designing proteins tailored to bind a small molecule of interest, and use it to make 'fluorescence-activating' proteins — biotechnological tools that have potential applications in biomedical research.

The functions of proteins are dictated by



ROBERTO A. CHICA

Figure 1 | Binding by design. Dou *et al.*[1] report a computational method for designing protein structures (known as β-barrels) that can bind any target molecule. They have used it to make small proteins that bind the molecule DFHBI, which fluoresces when bound. The crystal structure of one of the proteins in complex with DFHBI is shown here as a ribbon structure.

the specific three-dimensional structures into which they fold. One such structure is the β-barrel: a pleated β-sheet rolled into a cylindrical structure. This is frequently found in natural proteins whose function is to bind small ligand molecules[2]. So far, several challenges have prevented the *de novo* design of β-barrel proteins that can bind target small molecules. One issue is that the cylindrical shape of β-barrels requires the two edges of the original β-sheet to come together to form the base of the cylinder, a process that is complicated by the tendency of β-sheets to form intermolecular interactions that generate protein aggregates. Another is that these cylinders must be open at one end to allow the binding of a small molecule inside the barrel, which can cause substantial destabilization of the protein's folded structure. Thus, the design of ligand-binding β-barrel proteins requires a delicate balance between stability and ligand-binding activity.

Designing a cavity that is geometrically and chemically complementary to the small molecule of interest is just as challenging. This objective is complicated by the huge number of positions and orientations in space that the small molecule could adopt, and by the difficulty of identifying the exact combination of amino-acid residues that will make the necessary interactions with the ligand for specific binding. The latter point is particularly challenging given that there are some 10^{143} possible amino-acid sequences for proteins made up of 110 amino acids (as Dou and colleagues' β-barrel is). Thus, accurate and efficient computational procedures are required to find optimal solutions from such an astronomically large number of possibilities.

Dou *et al.* provide a solution to these problems. They started by devising general principles for designing stably folded β-barrel proteins of a predetermined size. They discovered that structural irregularities known as glycine kinks and β-bulges must be introduced into β-barrels to alleviate molecular strain and to maintain the continuous pattern of hydrogen bonds needed to form the cylindrical structure. Using their approach, they built computational models of 500 possible β-barrel 'backbones' and performed calculations to identify amino-acid sequences that would stabilize each backbone. The four designs that were predicted to be most stable were synthesized, and the authors observed that one of these folded into a monomeric β-barrel. Strikingly, the computationally predicted model and the experimentally determined structure were very similar, and the designed protein was highly stable.

The authors went on to design a β-barrel protein that binds DFHBI, a small molecule that fluoresces on binding. They used an innovative computational procedure that models a large number of poses (positions and orientations in space) of a small molecule and how they dock into a protein binding site while simultaneously optimizing protein sequences for binding activity. Through this approach, the authors generated 56 β-barrel designs that were predicted to fold stably and to bind tightly to DFHBI. Of these, 20 were found to be monomeric and soluble when tested experimentally, and exhibited some of the characteristic properties of β-sheets — indicating that they were potentially β-barrels. Two of those proteins bound DFHBI with moderate binding affinities (in the range of 13–50 micromolar).

Dou and colleagues next used an iterative procedure to further improve the binding affinity of their proteins. This included steps in which the X-ray crystal structures of two protein variants were used to guide additional rounds of computational design, and in which every amino-acid residue of another variant was systematically mutated to find changes that improved binding affinity. In this way, they identified three "mini-fluorescence-activating proteins" (Fig. 1) that bind DFHBI with submicromolar affinity and enhance its fluorescence both *in vitro* and *in vivo*. These designer proteins could be used to monitor gene expression and to track proteins in cells, as well as in biosensors that detect the presence of chemicals.

The development and application of this computational method for designing β-barrel proteins that bind small molecules is the first demonstration of the *de novo* design of both protein fold and function, a milestone in the field. Previous computational designs of ligand-binding proteins relied on building a binding cavity into a protein template found in nature[3], or one that had previously been created in the laboratory[4]. By contrast, Dou and co-workers have designed a β-barrel protein that has a shape distinct from those found in nature, and constructed a binding pocket that is specifically tailored to a target small molecule.

As noted earlier, the authors' initial designs needed further optimization to identify proteins that have sufficiently high binding affinities for potential applications. More-accurate predictions of protein structures are needed to eliminate the need for such fine-tuning. One way of achieving this might come from recognizing that proteins are not rigid molecules that adopt a single predominant structure — like all machines, proteins need to move to accomplish their tasks with high efficiency[5,6]. Indeed, ligand binding is often the trigger that causes a protein receptor to undergo a structural change enabling the transmission of a biological signal[7]. Computational methods for the rational design of proteins that undergo particular structural changes have recently been developed[8]. If these could be combined with Dou and colleagues' approach, it might be possible to access more-complex protein functions than were previously possible, opening the door to the on-demand creation of protein-based molecular machines. ■

Roberto A. Chica *is in the Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada.*
e-mail: rchica@uottawa.ca

1. Dou, J. *et al. Nature* **561**, 485–491 (2018).
2. LaLonde, J. M., Bernlohr, D. A. & Banaszak, L. J. *FASEB J.* **8**, 1240–1247 (1994).
3. Tinberg, C. E. *et al. Nature* **501**, 212–216 (2013).
4. Polizzi, N. F. *et al. Nature Chem.* **9**, 1157–1164 (2017).
5. Kerns, S. J. *et al. Nature Struct. Mol. Biol.* **22**, 124–131 (2015).
6. Catterall, W. A., Wisedchaisri, G. & Zheng, N. *Nature Chem. Biol.* **13**, 455–463 (2017).
7. Latorraca, N. R., Venkatakrishnan, A. J. & Dror, R. O. *Chem. Rev.* **117**, 139–155 (2017).
8. Davey, J. A., Damry, A. M., Goto, N. K. & Chica, R. A. *Nature Chem. Biol.* **13**, 1280–1285 (2017).

This article was published online on 12 September 2018.