De novo design of a fluorescence-activating α-barrel

Reviewed by Balz Marty – Report 03 – BIO-468

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Summary

The paper "De novo design of a fluorescence-activating β-barrel" [1] describes two breakthroughs in de novo protein design.

First, the authors solved the longstanding challenge of designing a β-barrel forming protein from scratch. They started out with a parametric backbone design approach. When this ultimately proved fruitless, they instead built β-barrel backbones starting from a 2D map, which characterizes the constraints on the peptide structure, and proceeded to generate 3D protein backbones computationally. A set of those designed proteins was expressed, and crystallography of one sample showed good agreement with the computationally derived structure.

Then, they used this approach again to design the first full de novo design of a small molecule binding protein. They computationally designed a β-barrel with a cave which can accommodate the small fluorescent compound DFHIB. As DFHIB does not fluoresce unless it is constrained in a particular conformation, they sought to design a binding partner which does so. They found several such β-barrels and analysed both binding and structure in vitro. Subsequently, they used a simulation-guided approach to find mutations that optimize the fluorescence function of the complex, whilst not jeopardizing the stability. Finally, they expressed the novel protein in vivo to find that it reliably binds and fluorescently activates DFHBI but that the complex does not reach the brightness of eGFP.

Limitations

As always, I did not find much to criticize in this article published in Nature.

Just one claim made by the authors appeared slightly curious to me. They discuss at length the problems they encountered when using a parametric approach to protein design. Summarizing this, they wrote in the abstract:

"We show that accurate de novo design of β-barrels requires considerable symmetry-breaking to achieve continuous hydrogen-bond connectivity and eliminate backbone strain."

However, to me - from what I read in the text – it is more of a hypothesis that the symmetry requirement makes the parametric approach entirely unsuitable.

Strengths

It is an impressive paper for what the authors achieved in this study: designing both the first full de novo β-barrel and small molecule binding protein. The potential implications for drug design are enormous if it was possible to design proteins which bind specific small molecule ligands.

What I liked particularly is the combination of both computational and more traditional wet lab methods. That is, computationally derived insights are extensively validated by other means.

Questions

There are some aspects that were not entirely clear of where simply I would like to have more background information.

The way I understood it, glycine is normally disfavoured in natural β-sheets. Yet, the authors almost seem to suggest that 'glycine kinks' are a requirement for forming stable β-barrels. Are there protein structures in nature more symmetric?

And how do the results generalize in terms of drug design? Can one design β-barrels to bind just about any small molecule target? I have not found any data about the selectivity of the binding. Are such β-barrels expected to have many off target effects or does their unique structure prevent that.

References

[1] J. Dou et al., 'De novo design of a fluorescence-activating β-barrel', Nature, vol. 561, no. 7724, Art. no. 7724, Sep. 2018, doi: 10.1038/s41586-018-0509-0.