Computational design of a modular protein sense-response system

A.A., Glasgow et al. – Reviewed by Balz Marty – Report – BIO-468

October 16, 2020

Summary

The paper "Computational design of a modular protein sense-response system" [1] describes a computational strategy for designing sensor-actuator proteins. It involves building small-molecule binding sites de novo into heterodimeric protein-protein interfaces. The two proteins associate in the presence of the ligand, which can be exploited to activate a dimeric reporter, whose subparts sit on the two proteins, respectively. Such a system promises to be flexible; in principle it could be designed to bind almost all small-molecule targets and it is (biological) output signal can be customized.

The authors chose to demonstrate their approach by designing such a chemically induced dimerization system (CID) to detect the metabolic compound farnesyl pyrophosphate (FPP). The computational approach comprises four steps:

(i) determining the geometry of the binding sites composed of three or four motive residues.
(ii) modelling these geometries into a set of heterodimeric protein-protein interfaces.
(iii) optimizing binding sites using flexible backbone design methods.
(iv) ranking individual designs for experimantal testing.

They proceeded by extensive testing of their reporter system. They expressed the most promising candidates in *Escherichia Coli* and characterized their signalling performance. Several rounds of (mostly targeted) mutagenesis improved the sensors' affinity for FPP. The crystal structure of one design was solved, which turned out to be in close agreement with the computational design.

Limitations

The authors are a bit quick to declare victory from my point of view. Although they showed just in a single instance that their computational approach for designing protein sense-response system works, they conclude:

"A previous computational analysis suggested that the appearance of pockets around artificially generated protein–protein interfaces may be an intrinsic geometric feature of protein structure [2], lending support to the idea that our approach is extensible to many other small molecules and interfaces. The design method presented here thus introduces a generalizable way to create synthetic sensing systems with different outputs that can be used in diverse biological contexts to respond to user-specified molecular signals."

Aggravating this situation, only two of the nine selected initial computational designs showed a robust signal, and both resulted from a single scaffold. This puts additional question marks behind the claim of generalizability.

In addition, their approach was not exclusively computational. The best performing protein sense-response system relied on a mutation identified by crystallography as potentially beneficial.

Moreover, the authors describe that crystallization occurred only in the presence of FPP, but one of the two resulting complexes did not contain FFP in the binding site. What an off-target binding could mean for the protein sense-response system is not addressed in the paper as far as I can see.

All in all, the approach does not look like a complete and foolproof recipe, that could be widely applied to almost any target, just yet.

Strengths

Although I am not convinced that the authors have yet shown beyond reasonable doubt that the computational system as such could be applied successfully to a whole range of targets , it is certainly a promising possibility. What I especially like is that in the case of FPP they could use just switch reporter signals and the system remained functional.

Another strong point is that they went to great lengths to optimize their reporter system using an ensemble of very different strategies. For me, the success of this optimization shows the value of combining computation with more traditional methods.

Questions

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

Why was this dimer approach chosen? Does it make selective signal transduction easier? Are there alternatives, where a single binding partner can produce the same effect? If yes, is it more difficult to generate such a system that generalizes to different ligands?

What is the relevance of protein sense-response systems? Is the goal to boost our understanding of such systems that occur naturally? Can they be used for biosensors? Or to improve the standard tool kit of traditional labs working in molecular biology?

References

[1] A. A. Glasgow et al., 'Computational design of a modular protein sense-response system', Science, vol. 366, no. 6468, pp. 1024–1028, Nov. 2019, doi: 10.1126/science.aax8780.

[2] M. Gao and J. Skolnick, 'The distribution of ligand-binding pockets around protein-protein interfaces suggests a general mechanism for pocket formation', Proc Natl Acad Sci U S A, vol. 109, no. 10, pp. 3784–3789, Mar. 2012, doi: 10.1073/pnas.1117768109.