MOLECULAR ENGINEERING

Designer binders

Researchers define rational design principles to make a small-molecule ligand-binding protein from scratch.

Small-molecule ligand-binding proteins are important tools for biology and biotechnology. Such tools can be created by raising antibodies against the molecule of interest or by directed evolution of an existing protein with a low affinity to the ligand to increase its binding power. But neither of these established approaches offers complete design flexibility or complete control over the binding interaction.

David Baker of the University of Washington and his colleagues recently established principles guiding the rational design of ligand-binding proteins. They identified three crucial properties for creating a successful designer binder: the existence of specific hydrogen bonding and van der Waals interactions between the protein binding site and ligand, good shape complementarity between the binding site and ligand, and a structurally 'preorganized' binding site.

They used computational tools to design an optimal binding site for the steroid ligand digoxigenin, positioning appropriate amino acid side chains around it to satisfy the three guiding design principles. They stationed the designed binding site in a set of receptive protein scaffold structures. To increase binding affinity, they further computationally optimized the residues surrounding the binding site. Then they evaluated the designs for binding affinity, shape complementarity and binding-site preorganization, and they selected 17 promising sequences for experimental characterization.

The researchers displayed the 17 designer binders on the surface of yeast and panned for binding to digoxigenin by flow cytometry. Of the 17, 2 bound digoxigenin, which the team confirmed by fluorescence polarization and isothermal titration calorimetry. Not stopping there, they optimized the top binder using site-saturation mutagenesis and yeast surface-display selection. Further, they used deep sequencing to map the effects on binding of single amino acid mutations at crucial positions. Implementing these results, they finally attained a highly specific binder with picomolar affinity for digoxigenin, a degree of affinity typically characteristic of antibodies.

Not only does the work offer new insights into protein-ligand interaction chemistry, the digoxigenin designer binder may also be a useful therapeutic tool. Whether the approach can be used to create other useful designer binders remains to be seen, but the outlook seems promising indeed.

Allison Doerr

RESEARCH PAPERS

Tinberg, C.E. *et al.* Computational design of ligand-binding proteins with high affinity and selectivity. *Nature* **501**, 212–216 (2013).