

Protein by Design: Computational Engineering of a Ligand-Binding Protein

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Protein-ligand interactions underlie a host of biological processes including cell signaling and antibody-antigen recognition, but a comprehensive understanding of the physiochemical properties dictating both high affinity and specificity of a protein for a particular ligand has been elusive. The ability to computationally design a protein with high affinity and selectivity for a given ligand represents a rigorous test of the understanding of the principles governing protein-ligand interactions and could potentially be used to design ligand-binding proteins with defined specificities for use in biosensors, therapeutics, and diagnostics. However, previous attempts at computational design of ligand-binding proteins have been largely unsuccessful as evidenced by discrepancies between computational models and structural and biophysical studies of designed proteins (Schrier *et al.*, 2009). Thus, current methods of designing ligand-binding proteins rely upon directed evolution of proteins with some measure of affinity for the target ligand and/or standard methods of raising antibodies against a target antigen. However, these methods do not allow complete control over binding interactions.

A new study from the laboratories of Drs. Barry Stoddard (Basic Sciences Division) and David Baker (University of Washington) as well as additional collaborators at UW and Rutgers University, published in *Nature* describes the first successful computational design and *in vitro* characterization of two ligand-binding proteins using a novel approach that may someday "open up a new strategy to create systems that combine the tumor targeting properties of antibodies with the therapeutic activity of traditional chemotherapy agents," says Dr. Stoddard.

The authors set out to design proteins with high affinity and selectivity for the steroid digoxigenin (DIG), a derivative of the cardiac glycoside digoxin, which is used in the treatment of heart disease. The authors' computational approach took into account several characteristics of naturally occurring ligand-binding sites: (1) energetically favorable van der Waals interactions and hydrogen bonding; (2) shape complementarity to the ligand; and (3) structural organization of the binding site prior to ligand binding. Using these criteria, the authors designed 17 DIG-binding proteins that were then experimentally characterized. Designed proteins were expressed on the surface of yeast cells (yeast surface display) and tested for DIG binding using fluorescence-activated cell sorting (FACS) after incubation with DIG-biotin derivatives and a streptavidin-conjugated fluorophore. Two of the

tested proteins, DIG5 and DIG10, bound DIG with affinities in the low-to-mid micromolar range, with DIG10 showing higher affinity towards DIG.

To further enhance the affinity of DIG10 for DIG, the authors performed saturation mutagenesis, testing the effects of every possible amino acid substitution on this interaction. This yielded the DIG10 variant DIG10.1, which displayed a 75-fold increase in affinity for DIG over DIG10. The authors next generated a binding fitness map of 39 residues designed to interface with DIG by testing a library of variant proteins with 1-3 amino acid substitutions at each of these positions for DIG affinity. DIG10.1 variants with increased affinity for DIG were subjected to high-throughput sequencing to identify the frequency of these mutations in FACS-sorted cells. Further selection led to DIG10.3, which displayed picomolar affinity towards DIG. Notably, the affinity of DIG10.3 for DIG was comparable to that of anti-digoxin antibodies.

In addition to DIG, DIG10.3 was also able to bind the steroids digitoxigenin, progesterone, and b-estradiol with affinities consistent with the loss of one, two, and three hydrogen bonds, respectively, between the molecule and binding pocket within the protein, indicating that these molecules bind the DIG10.3 binding pocket in the same orientation as DIG. Strikingly, the steroid specificity of DIG10.3 could be altered by the mutation hydrogen-bonding amino acid residues in its steroid binding pocket. These results indicate that the high specificity of DIG10.3 for DIG is generated through designed hydrogen bonds.

The researchers are now turning their computational approach to the design of proteins for cancer therapy. Says Dr. Stoddard, "We are now working hard on studying the structure and mechanism of chimeric antigen receptors (protein molecules that are expressed on the surface of engineered T-cells for immunotherapy) and hope to one day be able to use protein engineering to create new types of antitumor targeting receptors that act to direct reprogrammed T-cells and accompanying chemotherapeutic agents directly to individual tumor cells."

[Tinberg CE, Khare SD, Dou J, Doyle L, Nelson JW, Schena A, Jankowski W, Kalodimos CG, Johnsson K, Stoddard BL, Baker D.](#) 2013. Computational design of ligand-binding proteins with high affinity and selectivity. *Nature* 501(7466):212-6.

See also: [Schrier B, Stumpp C, Wiesner S, Höcker B.](#) 2009. Computational design of ligand binding is not a solved problem. *Proc Natl Acad Sci USA* 106(44):18491-6.

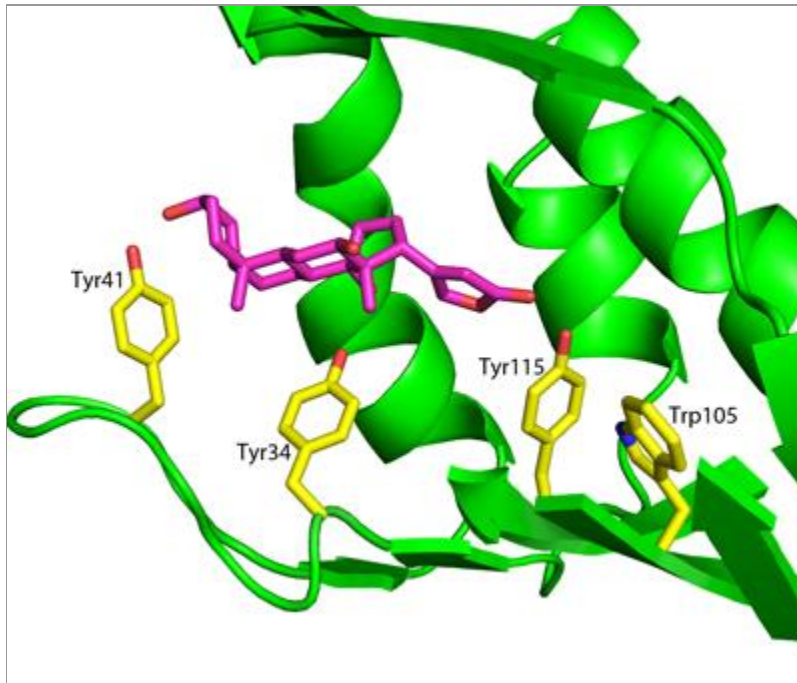


Image provided by Lindsey Doyle

Ribbon diagram of the active site of the computationally designed protein DIG10.3 in complex with its ligand digoxigenin (DIG). DIG-interacting residues of DIG10.3 are colored yellow and DIG is colored pink.