Computer-designed proteins recognize and bind small molecules

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From left to right, Christy Tinberg, Jiayi Dou and Jorgen Nelson work out some of their ideas on a whiteboard at the UW Protein Design Institute. Credit: Olga Khersonsky

Computer-designed proteins that can recognize and interact with small biological molecules are now a reality. Scientists have succeeded in creating a protein molecule that can be programmed to unite with three different steroids.

The achievement could have far wider ranging applications in medicine and other fields, according to the Protein Design Institute at the University of Washington.

"This is major step toward building proteins for use as biosensors or molecular sponges, or in synthetic biology—giving organisms new tools to perform a task," said one of the lead researchers, Christine E. Tinberg, a postdoctoral fellow in biochemistry at the UW.

The approach they took appears in the Sept. 4 online issue of *Nature*. Tinberg and Sagar D. Khare headed the study under the direction of David Baker, UW professor of biochemistry and Howard Hughes Medical Institute investigator. Khare is currently an assistant professor at Rutgers University.

Their *Nature* paper is accompanied by a News & Views commentary, "Computational biology: A recipe for ligand binding proteins." The commentator, Giovanna Ghirlanda of Arizona State University, wrote that the method developed "to design proteins with desired recognition sites could be revolutionary" because cell processes such as cell cross-talk, the production of gene products and the work of enzymes all depend on molecular recognition.

The scientific team overcame previously unsolved problems in building accurate protein-small molecule interfaces. Earlier attempts struggled with discrepancies between the computer plans and the structures of the actual molecules.

In conducting the study, the researchers learned general principles for engineering small molecule-binding proteins with strong attraction energies. Their findings open up the possibility that binding proteins could be created for many medical, industrial and environmental uses.

In medical diagnostics, for example, a rationally programmed protein might detect biomolecules found only in a specific disease state, such as an early-stage cancer. Other types of protein molecules might eventually be manufactured to treat an overdose or to block a poison. Remediation possibilities for these molecular workhorses could include trapping pollutants or capturing waste.

Tinberg explained that generation of novel small-molecule binding proteins currently consists of immunizing an animal to generate antibodies against a target protein, or directing the evolution of proteins in a laboratory to strengthen their affinity for the desired small-molecule.

"Neither of these methods allows complete control over the interactions involved in binding," she said.

In designing their molecules, the team sought to replicate properties of a naturally occurring protein binding site. These are: specific interactions that enforce a strong attraction with the desired small molecule, a receptive shape to accept the small molecule, and an orderly structure, prepared for occupancy. The exclusive, move-in ready set up reduces the energy penalty by preventing the protein from having to change shape to accept the small molecule. This is in contrast to a flexible site, which is more disordered in the absence of the small molecule and has to freeze into one state upon binding.

The scientists programmed the necessary protein-molecule interactions—and generated additional buttresses—mainly through the conformation and orientation of the binding site architecture.

"Our goal was a snug fit," Tinberg said.

The researchers adapted a computational tool called Rosetta developed

in the Baker lab to craft new proteins that would bind the steroid digoxigenin, which is related to the heart-disease medication digoxin. The drug can cause digestive problems, confusion, vision disturbances and heart beat irregularities. The difference between a helpful and a harmful dose is slight. At present patients receive antibodies directed at the molecule to correct excess amounts.

After generating many designs for digoxigenin-binders on a computer, the researchers chose 17 to synthesize in a lab. Experimental tests led the researchers to hone in on the protein they called DIG10. Further observations revealed that the binding activities of this protein were indeed mediated by its computer-designed interface, just as the researchers had intended.

To upgrade their overall design methods, the researchers then used next-generation deep gene sequencing to probe the effect of each amino acid molecular building block on binding fitness. Using this method, they were able to discover how various engineered genetic variations affect the designed protein's binding capabilities. The binding fitness map gave the researchers ideas for enhancing the binding affinity of the designed protein to the picomolar level, tighter than the nano-level.

The scientific team waited eagerly for the X-ray crystallography – a way of taking a picture of a molecule. It showed that the actual structures of two protein molecules matched at the atomic level with the computer-generated designs.

No longer did the researchers have to contend with the supposedly insurmountable roadblock – the mismatches between the design model and the protein generated in the lab.

Another goal of the work was to ensure that the designed proteins bound the small molecule target and not chemically related molecules. This

mistaken link-up could lead to side effects if the protein were used as a therapeutic. The researchers were pleased when their protein selected digoxigenin over three related steroids.

The scientists went on to redesign parts of the binding interface to change the protein molecule's preferences among three related steroids. The molecule could be reprogrammed to select either digitoxigenin (a relative of digoxin), progesterone (a female hormone), or B-estradiol (an estrogen-replacement drug.). The scientists manipulated the molecule's choices by altering its hydrogen bonding interactions.

The crystal structures of two designed proteins bound to digoxigenin have been deposited in the RCSB Protein Data Bank.

"By continually improving the methodology and with feedback from experimental results," the researchers noted in their paper, "computational protein design should provide an increasingly powerful approach to creating small molecule receptors for synthetic biology, therapeutic scavengers for toxic compounds, and robust binding domains for diagnostic devices."

The study is titled "Computational Design of Ligand Binding Proteins with High Affinity and Selectivity."

More information: www.nature.com/nature/journal/ … ull/nature12443.html

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https://phys.org/news/2013-09-computer-designed-proteins-small-molecules.html