

MARCH 02, 1999

## Better Binding through Chemistry

Many biological processes are triggered by the interaction, or binding, of molecules. So important is this motif in nature that numerous strategies have evolved to ensure the proper union of molecules. HHMI investigator Gerald Crabtree and colleagues at Stanford University have borrowed a trick used by microbes and cells in the immune system to engineer drug molecules that bind to their targets more effectively.

More importantly, says Crabtree, the approach also offers a new solution to the frustrating problem of developing small molecule drugs that can prevent two large proteins from binding to one another.

---

**"Small molecules have not been very successful at inhibiting protein-protein interactions because the protein surfaces are huge."**

— **Gerald R. Crabtree**

---

"Small molecules have not been very successful at inhibiting protein-protein interactions because the protein surfaces are huge," said Crabtree. "They are like molecular football fields, and using a small molecule to prevent these surfaces from coming together is like putting a pea down on a football field."

Crabtree and Stanford colleagues Roger Briesewitz, Gregory Ray and Thomas Wandless leveled the playing field by increasing the size of the small molecule inhibitor. They did this by chemically attaching the inhibitor to another small linker molecule that binds tightly to a much bigger "presenting" protein that exists naturally in the human body.

In essence, the combination of small drug molecule, linker and big protein allows the drug candidate to borrow the huge surface area of the presenting protein, making it appear to its target as if it is much bigger and stickier.

"Using a presenting protein is like bringing in a molecular wrecking ball instead of a pea," says Crabtree, because you "create a molecule with so much surface area that it now has a chance of out-competing the normal protein-protein interaction that you are trying to disrupt."

Crabtree, who has spent more than a decade deciphering the signals that turn on the immune system during a biological assault, and postdoctoral fellow

Roger Briesewitz got the idea for their approach while studying mutant bacteria that showed enhanced affinity of rapamycin for its target, tor, a protein involved in cell cycle regulation. Certain microbes block the proliferation of competing microbes by secreting rapamycin. The "drug" rapamycin achieves inhibition in two steps: First, it binds to a presenting protein, FKBP (for FK 506- binding protein), and then the drug-protein complex binds to the tor protein.

Remarkably, Crabtree says, all of the mutations that improved protein affinity were at the interface between the two proteins. No mutations were found in regions of either protein that directly bound to rapamycin. In thinking more about this unexpected result, Crabtree and his colleagues realized that their observation was related to the molecular mechanism by which small peptides, called antigens, turn on T cells. Antigens first bind to a much larger protein known as the major histocompatibility complex (MHC). X-ray crystallography studies have shown that both members of an antigen-MHC duo interact with the T-cell receptor.

"In fact, the antigen by itself has no affinity for the T-cell receptor, but when it's held by MHC, it has a very, very high affinity interaction," said Crabtree. "So, it's the presenting molecule that gives the high affinity."

"Similarly, the immune-suppressing drugs cyclosporin and FK506 have no special affinity for calcineurin's target protein in the human cell until they bind to large presenting proteins. In fact, when one mixes cyclosporin and calcineurin *in vitro*, there is no detectable binding. But when you have cyclosporin presented by a protein called cyclophilin, there is perhaps a million-fold increase in binding affinity to its target."

In a study published in the March 2, 1999, issue of the *Proceedings of the National Academy of Sciences*, the HHMI and Stanford scientists chose as their drug target a basic protein molecular subunit known as an "SH2 domain," which mediates protein-protein interactions that cause some diseases. This domain is regarded as a choice target for drug development because it is found in tyrosine kinases, enzymes that are critical components of the molecular machinery involved in cancer, osteoporosis, and inflammation. Despite its high profile among rational drug designers at pharmaceutical companies, the SH2 domain has proven to be an intractable drug target.

As their drug candidates, Crabtree and his colleagues chose peptides that contained the amino acid phosphotyrosine, which bind to the SH2 domain. The researchers hooked these peptides to a small linker molecule that sticks tightly to a human protein, FKBP.

Experiments with numerous peptide-linker combinations showed that certain combinations bound to the SH2 domain up to three times more tightly than did the peptide alone. These studies also demonstrated that it was possible to optimize binding by adjusting the linker segment so that it would place FKBP at just the right angle to produce the best fit with the target protein.

Medicinal chemists already take advantage of the fact that many drugs bind to blood-borne proteins such as serum albumin, which thereby allows small molecules to remain in circulation far longer than normal. Now, there is the potential to use another set of proteins to improve a drug's ability to bind to its target. "There are a great many candidate presenting proteins that exist inside and outside the cell, offering a wide variety of options for drug designers," says Crabtree.