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Putting Some Flexibility in Drug Design

Proteins are dynamic machines that bend, flex, and vibrate according to the demands of their daily tasks. They cannot sit still. But it turns out this range of motion may also reveal hidden weaknesses that can be targeted by therapeutic drugs.

Yet scientists at pharmaceutical and biotechnology companies typically rely on still images - molecular snapshots of the target protein - when designing new drugs that interfere with the activity of proteins. Howard Hughes Medical Institute (HHMI) researcher J. Andrew McCammon and his colleagues believe researchers may have better results if they factor a protein's inherent flexibility into the drug design process.

Over the last couple of years, McCammon and his colleagues have been perfecting a new technique that does just that. Although the method has a low-key name (relaxed complex scheme), it has already generated some impressive results. For example, scientists used key structural insights from this approach to pave the way for a recently approved drug for the treatment of HIV, as well as potential drugs to fight avian flu.

"If there is a structure of a potential protein target of a drug, the drug-design methods we are developing should be applicable."

- J. Andrew McCammon

Now, McCammon's group has used its method to develop three new drug candidates for African sleeping sickness, a disease that potentially endangers millions of people in Africa. Their results are reported in the October 27, 2008, early online edition of the *Proceedings of the National Academy of Sciences*.

"We wanted to come up with potential treatments for this dreadful disease," McCammon says. "Sleeping sickness has a high mortality rate, but people lose quality of life even before the disease reaches the terminal stage."

McCammon, who is an HHMI investigator at the University of California, San Diego, develops theoretical and computational methods for studying the function of biological molecules. By modeling the structure and dynamics of assemblies of these molecules, McCammon's group develops insights that help in the discovery of new pharmaceuticals. He says the improved method is likely to help in finding and designing drugs for many diseases, including influenza and cancer.

According to the World Health Organization, sleeping sickness threatens millions of people, mainly in sub-Saharan Africa. The disease is curable, but existing drugs are difficult to administer and have dangerous side effects. Without treatment, the disease is fatal.

African sleeping sickness is caused by *Trypanosoma brucei* parasites, which are transferred to humans or animals when they are bitten by infected tsetse flies. The disease gets its name from a late-stage symptom: nighttime insomnia and daytime slumber.

Kenneth Stuart, a coauthor of the *PNAS* article and a researcher at the Seattle Biomedical Research Institute and the University of Washington, says the team targeted its drug search to take advantage of a quirk in *T. brucei*'s physiology. To make the proteins it needs to survive, *T. brucei* heavily edits some of its RNA using large assemblies of proteins known as editosomes. Stuart's lab has shown that a component of the editosomes, called REL1 (RNA editing ligase 1), is present throughout the parasite life cycle. Without REL1, the parasites die and thus cannot cause sleeping sickness. Since REL1 is not present in mammals, the researchers thought it would make an excellent target for new sleeping sickness drugs.

One of the first steps in drug design is getting a clear picture of the target. In 2004, Stuart and his colleagues in Wim Hol's lab at the University of Washington obtained an atomic-resolution snapshot of REL1. They used a technique called x-ray crystallography to obtain a three-dimensional image of the protein. Such snapshots are frequently used to design drugs that specifically shut down a protein's activity.

McCammon and his colleagues used the knowledge of the three-dimensional image of REL1 in a novel way to design compounds that could interfere with REL1 and kill *T. brucei*. Typically, drug developers use computers to search for chemical compounds that block the protein's key working parts—a hint that the compounds will act specifically and have few side effects. The scientists sift through thousands of compounds in chemical libraries made especially for this purpose, and pick out those that recognize key sites in the protein's still image.

The problem with the conventional method is that proteins in cells do not sit still and are not rigid structures. Proteins are flexible. They perform dance-like movements driven by the thermal energy of their atoms, which

can prevent drug molecules from recognizing them. Hence, many drugs that look promising based on computational analysis ultimately fail to block their target's activity.

The team has already had considerable success with the approach. In 2007, the Food and Drug Administration granted accelerated approval for Isentress—a drug produced by Merck for the treatment of HIV infection, in combination with other therapies. Merck based their design of the new drug on data collected by McCammon and his colleagues detailing the flexibility of an essential HIV protein.

Adding information about protein flexibility to the search for new compounds to combat African sleeping sickness meant collecting much more data. Rommie Amaro, one of the first authors on the *PNAS* article, calculated REL1's movements using computer simulations and took thousands of three-dimensional snapshots of REL1 as it moved. These kinds of simulations are often used to understand the behavior of a protein, but their use to screen for potential drugs is new.

Testing thousands of compounds against each of thousands of snapshots is prohibitively expensive, but McCammon and his colleagues found that just like a dance, the movement of REL1 has a few essential poses that they could focus on in their drug design. The new application of a mathematical method allowed Amaro to pick these poses out from the thousands of snapshots she had generated. By testing compounds against these few poses, she could quickly take REL1's movements into account. “In this case, we didn't invent the wheel, we just made it a lot faster,” Amaro says.

Amaro used computational approaches to predict how well nearly 2,000 chemical compounds from a National Cancer Institute library would recognize the crystallographic snapshot of REL1. She then re-ranked the winners according to how well they recognized the essential poses of REL1. She focused on the top-ranking compounds whose size and composition matched the profile of an oral medication. In this way, Amaro and her colleagues narrowed down the compounds to just 14 candidates, which co-author Achim Schnauffer tested in the lab. Five of the fourteen turned out to inhibit REL1 activity. The traditional approach to drug design might not have uncovered all of these inhibitors and might have taken several more months, Amaro says.

To screen for side effects, Schnauffer tested the three REL1 inhibitors on two proteins that resemble REL1, including one human protein. In the test tube, the inhibitors affected the human protein much less than they did REL1.

Researchers have long been trying to use protein flexibility in drug design, and this method, makes it feasible. “We put racing wheels on the approach,” Amaro says.

The potential sleeping sickness drugs have yet to undergo extensive testing and refinement. In the next steps, the researchers will adjust the compounds to latch onto REL1 even more tightly. Those will then be tested on various cellular proteins to further screen for side effects.

“We are also working with influenza, tuberculosis, and other diseases,” McCammon says. “If there is a structure of a potential protein target of a drug, the drug-design methods we are developing should be applicable.”