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RESEARCH NEWS

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Computer Simulation Hints at New HIV Drug Target

For more than a year, researchers watched patiently as a few computer-simulated HIV protease molecules squirmed into more than 15,000 slightly different shapes. In real time, this contortion takes only a fraction of a second. In the end, however, this suspended animation paid off, as the simulations uncovered a potential new drug target to fight drug-resistant AIDS.

Howard Hughes Medical Institute (HHMI) scientists made the discovery while studying how one rare strain of HIV can evade a commonly prescribed class of drugs used to treat the virus that causes AIDS. The strain of HIV contained mutations that are often seen after failure of treatment with protease inhibitors, drugs that block the action of the enzyme protease and prevent the virus from making mature, infective copies of itself. When protease inhibitors fail — as they often do with a fast-mutating virus like HIV — new drug targets become vital.

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"Recognizing these variations in conformation — the three-dimensional arrangement of the amino acids that make up a protein — is the first step in identifying a new drug target," said Alex Perryman, first author of the study published early online in the journal *Biopolymers* on February 28, 2006. Perryman did the research when he was an HHMI predoctoral fellow in the lab of Andrew McCammon, an HHMI investigator in the Biomedical Sciences program at the University of California, San Diego. Perryman is now an Amgen postdoctoral fellow in the lab of Stephen Mayo, an HHMI

investigator at the California Institute of Technology.

"We hope these simulations will motivate the researchers in pharmaceutical or biotechnology companies to pursue the design of a new kind of inhibitor," Perryman said. "We are trying to lead other scientists toward a completely novel approach to fighting HIV infections."

In contrast to the colorful but static images on the covers of scientific journals, actual proteins are constantly quivering from the thermal motion of their atoms. Drug-binding sites that may be closed most of the time can be transiently exposed by an imperceptible atomic shudder. "This 'foot in the door' mechanism of drug binding and action is a relatively new way of thinking about things, arising from the greater appreciation of proteins as dynamic molecular machines," McCammon explained.

The drug cocktails now used in the treatment of HIV infection are referred to under the umbrella term, highly active antiretroviral therapy, or HAART therapy. Current HAART regimens generally include three antiretroviral drugs, usually two nucleoside analogs and either a protease inhibitor or a non-nucleoside reverse-transcriptase inhibitor. This drug regimen has, in some cases, been effective in controlling the progression of disease and prolonging survival. It has succeeded where other therapies have failed because HAART therapy makes it difficult for even a fast-mutating virus like HIV to develop resistance to all of the drugs at once.

But the specter of drug resistance is a real threat to each individual's therapy and to the long-term success of HAART for newly infected people. By one estimate, about 50 percent of patients receiving antiretroviral therapy in the United States harbor HIV viruses that are resistant to at least one of the available drugs. In fact, people with an infection typically have a diverse array of HIV mutants, some of which are resistant to antiviral drugs. Drug-resistant strains of HIV are being found more often in recently infected people, indicating that transmission of resistant strains is occurring at an increased frequency.

As a graduate student in McCammon's lab, Perryman initially set out to put the HIV protease enzyme in motion in a computer simulation, to catalog its many possible configurations. "HIV can wiggle and jiggle and move around into different shapes," he said. "We wanted to generate a bunch of conformations as possible targets for drug design."

Molecular dynamics simulations have already proved their value in steering drug design, McCammon said. "They helped in the discovery of the current HIV protease inhibitors, which bind to the active site of the enzyme. They were also key to the development of potential inhibitors of the third enzyme associated with HIV, the integrase enzyme.

“Other simulations by our group discovered an unknown binding site on the integrase enzyme, " McCammon explained. "Merck's new anti-integrase inhibitors include molecules that are designed to hit that site, in addition to a site that was known earlier from x-ray crystallography studies." These new inhibitors are moving into phase III human trials, he said.

For his first round of simulations, Perryman chose a rare double-mutant protease enzyme with two mutations in the 99 amino acids that make up each half of the enzyme. One of those mutations, V82F, is common, and it can emerge early during failure of therapy with most protease inhibitors. The other mutation, I84V, is frequently found after prolonged ineffective therapy with protease inhibitors and likely confers high-level resistance to most drugs in that class. The researchers wanted to compare the differences in the shapes of the non-mutated or wild-type enzyme, against which the drugs still worked well, and the drug-resistant double-mutant strain.

Perryman used a computer simulation program called AMBER that performs several different types of calculations. The x-ray crystal structure of the molecule is used as the input, and the various motions and shapes sampled are governed by Newtonian physics, the electric forces among atoms, the complementarity or clash of the different shapes that the enzyme takes, and penalties or bonuses for creating or relieving geometric strain.

The scientists depict the protease enzyme in a brightly colored cartoon, with features that resemble a fat cat face. From the front or the back, the identical halves of the enzyme have an ear and cheek protrusion on each side. (See illustration.) Frayed whiskers even appear to sprout from the bottom. The similarity to a face ends at the top of the molecule, where two flaps open to reveal a cavity. That is where enzymes and other proteins are cleaved into the parts necessary to assemble infectious virus particles. Structural studies of this cavity helped scientists find the original protease inhibitors. Other scientists are trying to design drugs to bind to the whiskers and or to lock down the flaps by binding to their top.

Perryman's first results, which were published in the April 1, 2004, issue of *Protein Science*, showed that the mechanism of drug resistance seemed to involve the motion of the flaps. More specifically, the double-mutant virus

displayed larger flap motions, especially at the tips. These larger movements seem to make it more difficult for the current drugs to function, since they must force the flaps to close and remain closed in order to prevent the enzyme from working. It probably takes more energy for the drugs to close the more mobile flaps of the mutant.

Perryman and his colleagues also observed that the flaps opened in a seesaw motion on each side, pinching the “cheek and “ear” together. That observation suggested to them that a small molecule might be able to wedge between the ear and cheek, blocking the flap opening.

"Some drugs act by binding to the active site of a target molecule, such as the site that an enzyme normally uses to catalyze reactions," McCammon said. "But increasingly, scientists are finding that other binding sites can be important. For HIV/AIDS, an important class of drugs called non-nucleotide inhibitors for reverse transcriptase typically bind at such alternate sites."

Once Perryman had a hypothesis to test in a second round of simulations — the proposed mechanics of the protease enzyme's nanomachinery—he used artificial restraints in the computer program to block the flaps from opening by expanding the gap between "ear" and "cheek." A new type of drug that binds there and controls flap motion could enhance the ability of the current protease inhibitors to bind to the active site, or it could offer a new way of inhibiting protease activity from afar by itself, Perryman suggested.

Now that he can prevent the simulated flaps from opening by using an artificial force, Perryman and co-author Jung-Hsin Lin of National Taiwan University hope to discover and develop real compounds that can regulate flap motion in this manner. Lin is currently assembling a team of researchers for this project. In the meantime, while Perryman is completing a postdoctoral fellowship, he is learning about protein engineering with Mayo, so that he can apply it to the design of therapeutic proteins as well as biomolecular electronics devices.