

Getting a Grip on AIDS

AIDS remains one of the most pressing U.S. public-health problems and is virtually out of control in less developed parts of the world. In South Africa, AIDS affects a third of the current populace of 43 million and is the leading cause of death, with no abatement in sight.

Because of their high cost, the drug “cocktails” that control HIV have been of little use in Africa. Even at their most successful, furthermore, these drugs aren’t a cure-all, and the quest for AIDS researchers remains, as it has been, to find a cure—not just therapeutic agents that manage the disease, but a knockout punch.

PSC physicist Marcela Madrid and Carnegie Mellon biologist Jonathan Lukin are contributing to this effort. Their computer simulations have revealed new understanding of an enzyme, HIV-1 reverse transcriptase (RT), that is an important target for AIDS drugs.

A Target for Drugs

Like many viruses, HIV carries its genetic information as RNA, which is single-stranded and must be converted to double-stranded DNA before the virus can reproduce. This is where RT comes into play. The enzyme takes its name from its function: It transcribes RNA to form the DNA version of HIV’s genome. This is “reverse” transcription because in most cells transcription goes the other direction—DNA to RNA. While the outlines of this RT copy-and-paste process are understood, the fine-grained molecular details remain a mystery.

What’s clear is if you find a foolproof way to stop RT from doing its job you will have cured AIDS. Several existing AIDS drugs work by binding to RT and blocking transcription. These therapies are less than fully effective, however, because HIV transcription is highly prone to error, giving the virus a protean ability to mutate and, thereby, to defeat drugs. For this reason, a detailed atom-by-atom picture of how RT does its job is a key goal of AIDS research.

A major step since the late 80s has been to deduce the structure of RT, called crystal structure since it’s obtained by crystallographic techniques. This work shows that RT’s active domain, where transcription takes place, is analogous to a hand, with subdomains that roughly represent a thumb, fingers and palm. Interestingly, two versions of this structure—one bound with DNA, one not—show a big difference: With DNA, the thumb is extended and open, making space for the DNA to fit into the palm; without DNA, the thumb is folded over to almost touch the fingers.

This was Madrid’s entry to HIV research. Her CRAY T3E simulations at PSC last year showed that removing the DNA from the open-thumb structure caused the thumb to close, in agreement with the closed-thumb crystal structure, indicating this is RT’s “native-state.” These simulations, furthermore, support thinking that RT’s movable thumb may be a key to transcription, allowing the enzyme to slide along the RNA strand as it adds bases one-by-one to form DNA.

Concerted Motion

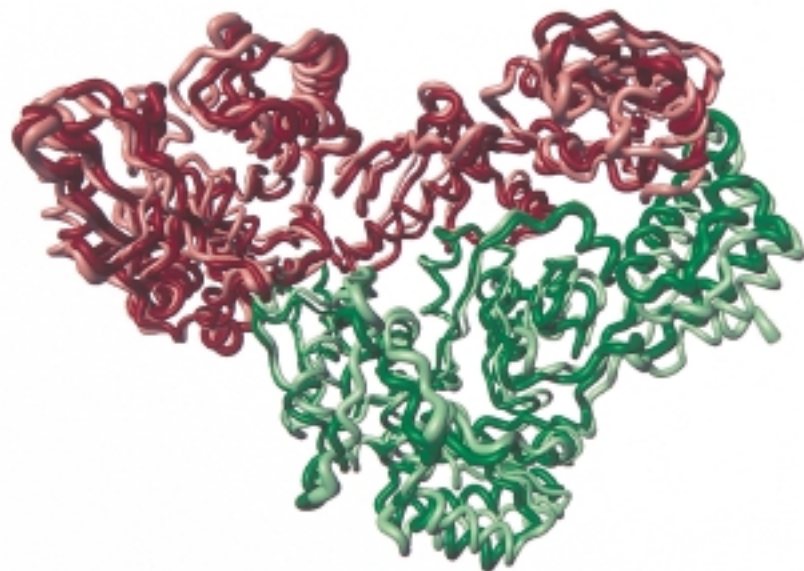
Along with being involved in transcription, the joint-like flexibility of the RT thumb may be a factor in how one class of AIDS drugs inhibits HIV reproduction. It’s believed that these drugs lodge in the palm of RT and lock the thumb in the open position. “We want to understand RT’s flexibility,” says Madrid, “when it’s by itself, when it’s bound with DNA and when it’s bound with drugs.”

With this objective, Madrid and Lukin used the SGI Origin 2000 at NCSA in Illinois to simulate RT structures with and without DNA. By adding 37,000 water molecules to immerse the RT molecule in a water bath, these simulations had a degree of realism missing from the prior study. The plan was to compare the fluctuations of each atom as shown in molecular dynamics movies to the crystal structures, which deduce atomic position according to electron density, and therefore include a measure—called the crystallographic B-factor—that represents uncertainty in the position of an atom, evidence for motion.



▲ Jonathan Lukin and Marcela Madrid.

[Toward Solving the
Puzzle of HIV Transcription]



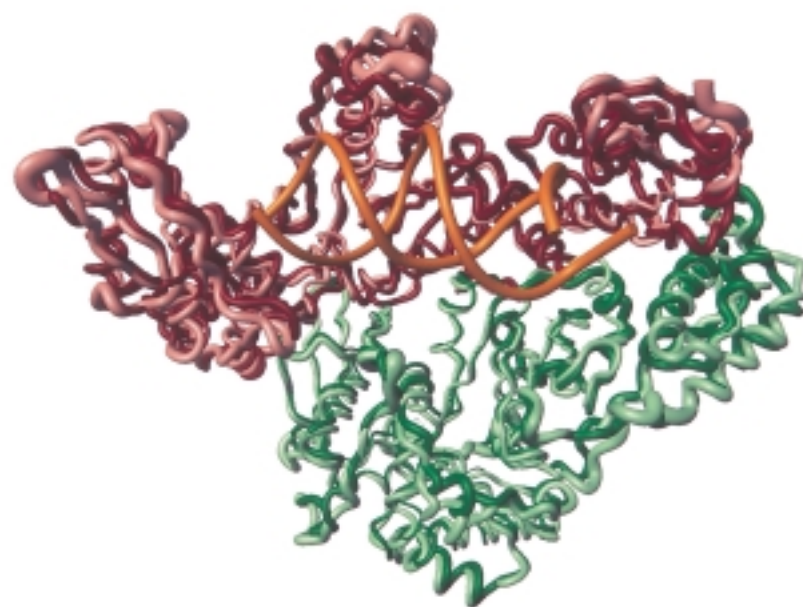
Flexibility of HIV-1 Reverse Transcriptase

The crystal structure of RT compared to the simulated structure without (left) and with DNA (orange). Coil thickness corresponds to the crystallographic B-factors and simulated movements. The entire molecule is represented, with the active-site subunit shown in red (crystal structure) and pink (simulation) and RT's other subunit in corresponding dark and light green. Analysis of these simulations finds a pattern of motions among regions in concert with each other.

For the structure without DNA, the results showed good correlation between the simulations and B-factors, giving confidence in the accuracy of the simulation. For the structure with DNA, the simulation showed greater flexibility in the tips of the thumb and fingers than suggested by the crystal structure, and also more flexibility than the structure without DNA. This simulation showed, furthermore, that the thumb and fingers and a binding pocket in the palm are moving at the same time—information that can't be obtained experimentally.

Though well short of a complete solution to the HIV transcription process, this dynamic picture complements the crystal structures and pulls together much of the available information into a consistent whole, offering a blueprint for future work. In their next project, Madrid and Lukin plan to simulate RT structures that include drug molecules that inhibit transcription. "We'll be looking for clues," says Madrid, "to see how the drugs work."

More information: <http://www.psc.edu/science/madrid2000.html>



► A Handy Enzyme

This ribbon representation of the RT active domain illustrates its hand-like structure, showing fingers (blue), palm (pink) and thumb (green). The active site (red atoms), where DNA is elongated, is in the palm region. Also shown is an RT-inhibitor drug (yellow) in the pocket where it binds.

