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## How HIV Evades AZT

HHMI researchers have used x-ray crystallography to produce a three-dimensional picture of a key AIDS virus enzyme. The structure reveals details that clarify how HIV mutations render the retrovirus resistant to antiviral drugs such as AZT.

Once HIV invades a human cell, reverse transcriptase (RT), one of three enzymes used by HIV, reads the retrovirus's genetic material, RNA, and synthesizes DNA, which codes for viral proteins that commandeer the infected cell's protein-making machinery. In an article published in the November 27, 1998, issue of the journal *Science*, Stephen C. Harrison, an HHMI investigator at Children's Hospital in Boston, and his colleagues from Harvard University, describe the active structure of RT.

In order to capture RT in an active form, Harrison knew that the enzyme needed to be constrained, so he approached Harvard chemists, Gregory Verdine and his postdoctoral fellow Huifang Huang, and asked them to tether RT to DNA. The chemists modified both DNA and the RT protein so that the two would be attached by a permanent chemical bond. "The really clever new trick that helped us trap a catalytically active complex was this modified chemistry," Harrison says.

The first images of the structure of RT were produced in the early 1990s by HHMI investigator Thomas A. Steitz at Yale University. Steitz's research team used a catalytically-inactive form of the protein in their studies. Steitz and others showed that RT and other members of the DNA polymerase family of enzymes are composed of subunits that resemble fingers, a palm, and a thumb. In the RT found in HIV, DNA lies across this hand, stretching from where the wrist would be toward the metaphorical pinkie. The structure shows that active RT looks like a slightly clenched hand, with the thumb almost touching the finger nearest to it.

Most drugs that target RT mimic nucleotides. When the drugs are incorporated into DNA strands in the place of normal nucleotides, the strands stop growing, and this effectively stops viral replication. Mutations that confer resistance to AZT and other nucleotide analogs occur in the pocket between the thumb and forefinger where the nucleotide binds. The bending of the fingers that is clearly visible in the newly solved structure clarifies how mutations alter binding to the DNA nucleotide or to the nucleotide analog antiviral drugs such as AZT. Harrison's group shows that these mutations prevent the nucleotide analog drugs from binding to the enzyme. These

mutations do not prevent normal nucleotides from binding, however, which allows RT to continue making DNA for the virus. "Because of very elegant chemistry accomplished by Verdine and Huang, we've been able to trap an informative complex that allows us to understand much better the resistance mutations," Harrison says.

Harrison says he is also excited about the research because the structure of the catalytically-active form of RT may reveal general principles about how viral polymerases work.