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An HIV Enzyme with a Flair for the Acrobatic

Call it the bullet train of enzymes. Howard Hughes Medical Institute (HHMI) researchers have found that HIV reverse transcriptase exhibits a stunning display of enzymatic dynamics as it zooms back and forth on the very DNA it is building. Each of the DNA's two strands serves as a rail, with reverse transcriptase - an enzyme crucial to HIV's replication and survival - racing to the end of the rails so it can continue to extend them.

HHMI investigator Xiaowei Zhuang and colleagues at Harvard and the National Cancer Institute discovered this racing motion by watching HIV reverse transcriptase in real time using a method called fluorescence resonance energy transfer (FRET). They report the findings in the November 14, 2008, issue of *Science*.

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— **Xiaowei Zhuang**

We discovered that HIV reverse transcriptase is extremely dynamic, exhibiting both large scale translational and large scale orientational movements, said Zhuang.

The work also provides new insights into how an important class of anti-HIV drugs, called non-nucleoside reverse transcriptase inhibitors (NNRTIs), block some functions of HIV reverse transcriptase. An editorial accompanying the article suggests that the new insights will inspire ideas for even better anti-HIV drugs. We saw effects of NNRTIs that nobody knew about, said Zhuang.

HIV reverse transcriptase has a big job. It transforms single strands of RNA encoding the HIV genome into double-stranded DNA that can integrate into the genome of the host cells of an infected person. To complete this job, HIV reverse transcriptase grabs onto the RNA - a single rail - and builds a parallel rail out of DNA nucleotides. Later, the enzyme chops up the RNA and builds a second, final, DNA rail.

As the enzyme goes about its work, it drops off the lengthening complex often, after placing only a few to a few hundred bases of DNA. Because the HIV genome is 10,000 base-pairs long, the enzyme must frequently re-find its place on the nascent strand of DNA that it is building. Researchers assumed that HIV reverse transcriptase found the working end of the DNA by blind luck.

But through FRET analysis, which uses fluorescent dyes to track precise movements of molecules, Zhuang and her colleagues saw something else: the enzyme grabs onto the middle of the DNA molecule and slides to the end to continue its work. Instead of searching for the right place in a three-dimensional space, sliding allows the enzyme to search along a single dimension. We think this may enhance the enzyme's efficiency. said Zhuang.

However, because the middle of the double-stranded DNA does not have a directional bias, once the enzyme binds and slides to the end of the growing DNA molecule, it may be facing backward, caboose-first instead of engine-first. This indeed happens and under this circumstance, HIV reverse transcriptase flips 180 degrees. If the enzyme binds in the wrong orientation, and then does its sliding search, it can self-correct and flip into the right orientation, said Zhuang.

Zhuang and her colleagues first reported the flipping behavior in the May 8, 2008, issue of the journal *Nature*. Switching analogies, she likens the move to that of a gymnast on the parallel bars. With hands grabbing a bar, the gymnast swings her two legs between opposite directions. HIV reverse transcriptase can do the same thing, and then perform different tasks depending on its orientation. Facing one direction, it adds nucleotides to extend the DNA molecule. Facing the other, it destroys the RNA template strand it no longer needs.

The orientation determines the function, said Zhuang. When it needs one function, it binds in one orientation. When it needs another, it binds in the other orientation. When it needs both functions, it flips between them.

Together, the *Science* and *Nature* papers paint a portrait of an acrobatic protein capable of moves no one had seen in an enzyme before. Zhuang thinks such dynamic moves might play a role in increasing the efficiency of a multi-functional enzyme like HIV reverse transcriptase. The enzyme has several different tasks to perform, and such dynamic flexibility may help the enzyme to adjust quickly into the proper binding configurations to get the jobs done more quickly.

As for the NNRTIs, they reduce the efficiency of the enzyme by making HIV reverse transcriptase flip and slide into the wrong binding configurations, turning the enzyme into a twitchy, fidgety worker. The NNRTI findings were a byproduct of our work observing the interactions of HIV reverse transcriptase with its substrates, said Zhuang. But I'm hopeful the discoveries will help other researchers design more effective anti-HIV medicines.