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## Scientists Watch New HIV Target Flip Out

HIV flips for membranes. That's the conclusion of new research from Howard Hughes Medical Institute investigator Michael F. Summers and colleagues who have identified a new drug target that could defeat HIV's rapid evolution, the main mechanism of drug resistance.

It could be an important antiviral target, said Summers, who is at the University of Maryland, Baltimore County. Summers and his colleagues published their article in the July 25, 2006, issue of the *Proceedings of the National Academy of Sciences*.

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— **Michael F. Summers**

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The work completes the puzzle of how new HIV particles assemble at the cell membrane before popping out of the cell to infect others. The question is, how is this main HIV protein, called Gag, smart enough to know which membrane to go to? said Summers. Cells are full of membranes - at the nucleus and other subcellular structures - and HIV only assembles at specific membranes (usually the outer plasma membrane). If it migrates to the wrong membrane, it won't assemble and reproduce.

It turns out that Gag proteins surreptitiously scan the cell's internal signposts. When they find the right address on a particular location of the outer membrane, they latch onto the signpost. Thousands of other Gag proteins join in to assemble a new viral shell. Advanced nuclear magnetic resonance (NMR) studies from Summers' group reveal exactly how this happens.

The research was prompted by earlier work from National Cancer Institute scientist Eric Freed, who discovered that one of the cellular signposts, phosphatidylinositol (PI) 4,5-bisphosphate, or PIP2, was essential for HIV assembly and replication. But Freed didn't know exactly what role PIP2 played. He just knew that if you remove that signpost from the plasma

membrane, HIV didn't assemble at that membrane, said Summers. Furthermore, if that signpost was artificially moved to a different internal membrane, HIV assembly was re-targeted to that membrane.

Scientists also knew that a specific end of the virus's Gag protein, called the matrix domain, or just matrix, played a role in HIV's assembly at the cellular membrane. We had the two pieces but we didn't know how they worked together, said Summers.

His NMR studies showed that PIP2 can bind to matrix, and indicate that when matrix bumps into PIP2, a two-step binding process locks Gag to the cellular membrane. First, matrix grabs onto PIP2, which serves as a bridge between matrix and the membrane. This binding triggers matrix to flip out and expose a fatty acid tail that drives into the membrane. A tight embrace results: one arm of PIP2 locks deep into matrix, and the tail of matrix juts into the membrane.

Normally, the tail of the matrix, called a myristyl group, is buried deep inside the protein where it can't bind to the membrane. But once matrix binds to PIP2, the myristyl group flips out and attaches to the membrane, said Summers. It's a beautifully simple mechanism for getting HIV proteins to bind only to membranes that display the right signpost.

The family of PIP molecules has drawn intense interest as cells' internal addressing system. Like molecular zip codes, various PIP molecules direct proteins to their proper locations. Summers' new work exquisitely shows how HIV hijacks this system.

Street addresses are a very good analogy, said Summers. Until now, though, no one knew how HIV proteins found the right address. The virus is very crafty in how it uses the cell's own machinery to help it reproduce.

Summers imaged the interaction of Gag and PIP2 with nuclear magnetic resonance (NMR) technology. Similar to magnetic resonance imaging machines found in hospitals, NMR scanners pulse a sample with radio waves, which causes all of the hydrogen atoms to line up in one direction. Between pulses, the hydrogen atoms spin back to their original orientation, releasing energy. NMR-linked computers interpret the energy signature, which is used to calculate a three-dimensional picture of the molecules.

The tight binding between the two molecules presents an enticing new drug target. A drug that binds to PIP2 could block Gag from attaching to the cellular membrane and assembling into mature HIV virions. Even better, Summers said that such a drug might be immune to new HIV mutations. While most of the proteins in the HIV virus mutate quickly, decreasing the effectiveness of the various anti-viral drugs on the market, the segment of matrix that binds to PIP2 does not mutate much. It's virtually the same across all strains of HIV. It's unusual for proteins in HIV to be conserved like this, said Summers. It suggests that, if a drug could block PIP2 and matrix binding, the drug would continue to work even as the rest of HIV mutates.