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Key HIV Protein Structure Solved

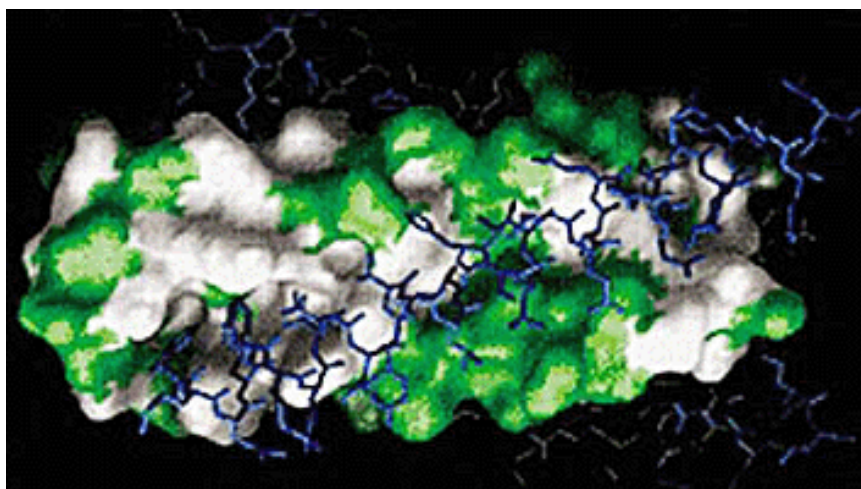


Image Title: Two teams of HHMI researchers determined the structure of gp41, a protein fragment from the surface of HIV that penetrates a cell's membrane like a sewing-machine needle. This allows the virus to "fuse" with the membrane of the cell and gain access to the cell's reproductive machinery. - Kim Laboratory/HHMI at the Whitehead Institute for Biomedical Research

Two Hughes investigators, working in parallel, advanced our understanding of AIDS when they discovered the way HIV, the virus that causes AIDS, penetrates a cell.

They determined the precise structure of a protein fragment from the surface of HIV that penetrates a cell's membrane like a sewing-machine needle. This allows the virus to "fuse" with the membrane of the cell and gain access to the cell's reproductive machinery.

The structure of the protein, gp41, was discovered by two independent teams one led by Peter Kim, a Hughes investigator at the Whitehead Institute for Biomedical Research and MIT, and a second led by Don Wiley, a Hughes investigator at Harvard University and Children's Hospital, whose team included Hughes investigator Stephen Harrison, also at Harvard and Children's Hospital.

The pictures of gp41 produced by Kim and Wiley show that it is similar to proteins on the surface of other viruses, an observation that opens new avenues for basic research and new possibilities for antiviral drugs.

"Fusion is enormously important biologically," said Robert Lamb, a Hughes investigator and virologist at Northwestern University. "Kim and Wiley have helped move the search for drugs against HIV from the theoretical to the practical."

Both labs began their route to this discovery not with studies of HIV but with meticulous examination of the influenza virus. HIV and influenza both display proteins that stand on the outer surface of the viral membrane like armed torpedoes poised to fire. As influenza approaches a host cell, it latches on to a ubiquitous substance found on the cell surface. HIV, however, requires a specific docking molecule, called CD4, to grab hold.

Scientists now know that after both of these viruses have "docked" with a host cell, they use remarkably similar strategies to make certain that the genetic information they carry is successfully propagated. Influenza's path to penetration is especially devious, while HIV using a similar protein takes a more straightforward route.

Once influenza has hitched its ride, the afflicted cell soon realizes that an interloper is piggybacking on its outer surface, and it begins to swallow the intruder by enveloping the virus in a bubble that forms on the cell's surface. When the bubble virus and all is brought inside, it would appear that the cell has won the battle. The cell then pumps acid into the bubble, which lowers the pH inside in an attempt to kill the contents.

Using the lower pH provided by the cell, influenza administers the coup de grace. The pH change actually triggers the viral

firing mechanism, and the protein torpedoes the bubble wall. The virus, already inside the cell, now dumps its genes into the cell's interior.

HIV's modus operandi is similar but simpler. Researchers believe that it is HIV's interaction with CD4 or other cellular proteins rather than lowering pH that triggers changes in the shape of the gp41 protein and allows HIV to enter the cell.

In 1981, Wiley and colleagues in England solved the structure of the influenza virus' needle-like surface protein. "There were three helices wrapped around each other," said Wiley. "And when we lowered the pH, everything changed radically. Like a jack-in-the-box," the virus' surface protein "pops out" and penetrates the bubble membrane.

Kim's contribution to the field began in earnest in 1993, when he showed how influenza's needle-like mechanism works: Part of the protein that normally lies limp twists itself into a helix and acts as a lever to

catapult the rest. Confirmed and expanded upon by Wiley in 1994, the ideas contributed by the two scientists have become the model for the "spring-loaded" theory currently used to explain viral fusion by influenza.

When the structure of the influenza surface protein was published in 1981, Wiley and others surmised that other viruses might have similar structures that would make good drug targets.

With the discovery of HIV not long afterward, Wiley didn't have to wait long to test his ideas on an important new viral killer. Obtaining structures of HIV's surface proteins, however, proved extremely difficult because the proteins are literally sugar-coated. The more sugar molecules that are attached to the protein, the harder it is to obtain good crystals to analyze with X-ray diffraction. The influenza virus had seven sugar chains dangling off the surface protein. A similar protein in HIV has about 27 of these chains, said Wiley. "That makes it hard."

Kim tackled HIV with an approach that had brought him success with influenza. "Instead of trying to crystallize the whole thing," he said, "we chopped the protein into bits" and tried to reassemble enough of the pieces to see how the protein looked in the living virus. "We call it 'protein dissection,'" said Kim.

In the meantime, Wiley's team also chopped up the protein, but it used genetic engineering to add a section of similarly shaped protein from yeast. The extra yeast protein stabilized the HIV protein fragments enough to allow them to obtain a crystal structure.

Kim's results were published in the April 18, 1997, issue of *Cell*; Wiley's in the May 22, 1997, issue of *Nature*. The results of both teams were touted in an article that appeared on the front page of *The New York Times*. Both studies show that the HIV structure strongly resembles that of the influenza protein, with the same needle-like jabber. "This spring-loaded mechanism may be much more general than just the flu virus," said Kim. "Understanding the relationship between structure and mechanism is what you want to do."

Lamb adds that knowing the structure of the HIV protein, even though it is similar to those of other viruses, is "more than just a coda. You need the specific X-ray coordinates of the HIV protein if you want any hope of making drugs that block its function."

That hope hangs in the air. By 1995, other researchers had shown that two protein fragments called 'peptides' of HIV gp41 can inhibit HIV infection. Unfortunately, said Wiley, "peptides make lousy drugs" because they are digested in the gut and therefore cannot be taken orally. What are needed are small molecules that can mimic the peptides' activity.

Kim has his eye on one pocket in the HIV protein as a potential drug target. Wiley fears that finding a drug to fit in that pocket will not be so simple. "People do design drugs based on structure," he said, "but they work only in less than one case out of a thousand." On the other hand, Kim points out,

there are no drugs that specifically attack the gp41 protein, so if the long shot pays off it could have great therapeutic impact.

The discoveries raise many important questions for scientists trying to understand HIV. What does the structure of gp120, the other HIV surface protein, look like? How does gp120 interact with CD4 to allow HIV to bind a cell surface in the first place? Finally, does the HIV gp41 protein also change shape after HIV makes contact with CD4?

Kim and Wiley are pursuing these questions independently, continuing their sometimes intense, always respectful rivalry. The pair's different styles come out even in brief conversations in their Cambridge offices, which are barely a mile apart. Wiley roams the floor, contorting himself into yoga-like positions to get across the nuances of a complex biological structure. Kim is more buttoned-down, preferring to intertwine his fingers to show how the three helices of gp41 fit together.

Wiley credits Kim with an impressive all-at-once entry into the field of membrane fusion. "He does some clever experiments, there's no doubt about it." Wiley, according to Kim, "has an amazing nose for important biological problems that can be solved through structural analysis."

Both are ecstatic to have solved this much of the gp41 structure. "You can now do all sorts of tricks that you can only do after seeing the structure," said Wiley. "It's unbearably exciting."