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First Contact

In a long-awaited series of articles, HHMI researchers and their colleagues report the three-dimensional structure of the HIV-1 protein that makes first contact with human cells.

HIV-1's outer coat is studded with proteins that the virus deploys when it latches onto human cells. Researchers in academia and industry have studied these proteins in the hopes of uncovering a potential weak spot that can be used in a vaccine or exploited as a drug target. HIV-1 surface proteins are of paramount importance because they mediate attachment of the virus to its target cells.

Chief among those surface proteins is glycoprotein 120 (gp120), which pokes out from the surface of HIV-1. When the virus encounters a lymphocyte that bears the protein CD4 on its surface, gp120 docks with that lymphocyte. The virus also must bind to a chemokine receptor in order to initiate infection.

Once the virus is attached to both CD4 and a chemokine receptor, it then fuses with the host cell's outer membrane—beginning the process of viral replication. The gp120-CD4 interaction is generally thought to be one of the crucial steps in HIV-1 infection.

"We have visualized gp120 binding to CD4 in the presence of a neutralizing antibody," said Wayne Hendrickson, an HHMI investigator at Columbia University. "The gp120-CD4 interaction is critical for

positioning HIV-1 onto the target cell, which sets the stage for infection."

Hendrickson and Peter Kwong of Columbia University collaborated on the research with Richard Wyatt and Joseph Sodroski at the Dana Farber Cancer Institute and Raymond Sweet at SmithKline Beecham Pharmaceuticals. The research team published two articles in the June 18, 1998, issue of *Nature* and another article in the June 19, 1998 issue of *Science*.

Last year, two teams of HHMI researchers solved the crystal structure of gp41, an HIV-1 coat protein that harpoons T lymphocytes. For the last decade, though, research teams around the world have tried in vain to produce protein crystals of gp120 that would allow them to use X-ray diffraction techniques to determine the molecule's shape.

The chemical composition of the gp120 molecule was one of the biggest obstacles faced by researchers. Its inherent flexibility — due to a carbohydrate coat and flexible loop regions — makes it a difficult candidate for crystallography. X-ray crystallography works better when protein crystals form rigid, regularly repeating lattices. "More than half of gp120's molecular weight is due to the presence of sugars," Hendrickson said. Sugars form an unstable target, he noted, so they are best not included.

Wyatt and Sodroski, who have studied gp120's function in great detail by blocking the protein's binding sites with various antibodies, supplied many variant forms of gp120. Kwong, a postdoctoral fellow in Hendrickson's lab who began the gp120 project eight years ago, devised several ingenious biochemical techniques to shave off roughly 90 percent of the sugar from the gp120 protein. Sodroski's team with input from Hendrickson's group pruned away

flexible regions of gp120 to produce a crystal best suited to the rigid specifications of crystallography.

Their efforts finally paid off with crystals of gp120 complexed with CD4 and a neutralizing antibody. With crystals in hand, the team was able to bombard the molecules with X-rays produced at HHMI-supported Beamline X4A at the

National Synchrotron Light Source at Brookhaven National Laboratory.

The data from the X-ray studies show many unexpected features of the gp120-CD4 interaction, including a cavity-laden gp120-CD4 interface and the presence of a conserved binding site for the chemokine

receptor. Furthermore, the structures show that when gp120 changes shape upon binding CD4, the change unveils a binding site for antibodies on gp120. From looking at the structures, we can tell that there are whole regions of gp120 that the immune system never sees, said Hendrickson.

The researchers note in the *Nature* article: "The results provide a framework for understanding how HIV-1 gains entry to cells and could guide efforts to intervene."

Hendrickson acknowledges that the structures offer as many questions as they answer. He does believe, however, that these studies should clear the way for design of new drugs based on the structure of the gp120-CD4 interaction. "Because we have atomic detail of the interaction between CD4 and gp120, structure-based drug design becomes a real possibility," Hendrickson said.

"There's obviously a lot of interest in the structure of gp120 because it has been a crucial target for some of the vaccines already in clinical trials," said Hendrickson. "We believe that by having a better idea of what the antigenic surface is on gp120, we have a far better chance of being able to design the appropriate gp120 molecules for use in vaccines."