Tricky protein may help HIV vaccine development

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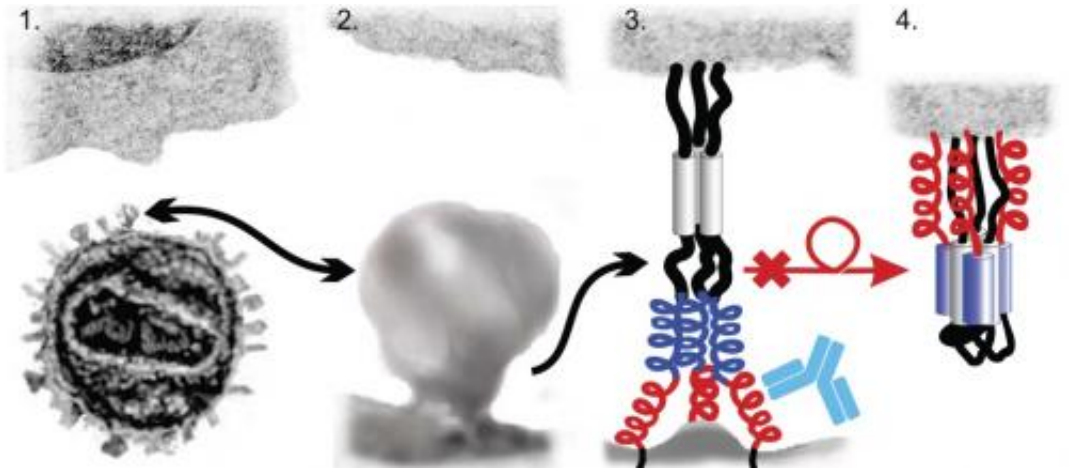


This illustration shows how the envelope proteins covering the surface of an HIV virion (1, 2) bind to a host cell (3, 4). The trimeric MPER region of gp41 is shown in red and can be disabled by antibodies, shown in light blue. Credit: Marian Miller

Duke scientists have taken aim at what may be an Achilles' heel of the HIV virus.

Combining expertise in biochemistry, immunology and advanced computation, researchers at Duke University have determined the structure of a key part of the HIV envelope protein, the gp41 membrane proximal external region (MPER), which previously eluded detailed structural description.

The research will help focus HIV vaccine development efforts, which have tried for decades to slow the spread of a virus that currently infects more than 33 million people and has killed 30 million more. The team reported the findings online in the Jan. 13 early edition of *Proceedings of the National Academy of Sciences*.

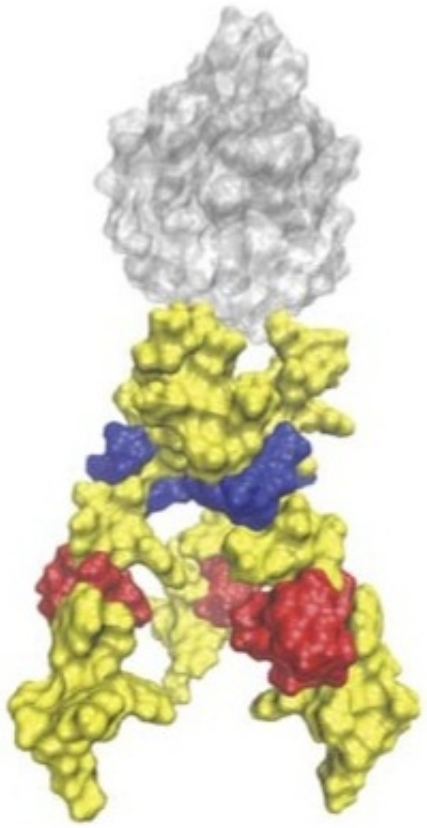
"One reason vaccine development is such a difficult problem is that HIV is exceptionally good at evading the immune system," said Bruce Donald, an author and professor in Duke's computer science and biochemistry departments. "The virus has all these devious strategies to hide from the immune system."

One of those strategies is a dramatic structural transformation that the virus undergoes when it fuses to a host cell. The envelope protein complex is a structure that protrudes from HIV's membrane and carries out the infection of healthy host cells. Scientists have long targeted this complex for vaccine development, specifically its three copies of a protein called gp41 and closely associated partner protein gp120.

The authors said they think about a particular region of gp41, called MPER, as an Achilles' heel of vulnerability.

"The attractiveness of this region is that, number one, it is relatively conserved," said Leonard Spicer, senior author and a professor of biochemistry and radiology. In a virus as genetically variable as HIV, a successful vaccine must act on a region that will be conserved, or similar across subtypes of the virus.

"Second, this region has two particular sequences of amino acids that code for the binding of important broadly neutralizing antibodies," said Spicer. The HIV envelope region near the virus membrane is the spot where some of the most effective antibodies found in HIV patients bind and disable the virus.



Three legs of the critical gp41 HIV protein are shown in yellow. Blue and red show where antibodies can bind and neutralize the virus when the legs are extended. Credit: Patrick Reardon, Leonard Spicer

When the virus fuses to a host cell, the HIV envelope protein transitions through at least three separate stages. Its pre- and post-fusion states are stable and have been well studied, but the intermediate step—when the protein actually makes contact with the host cell—is dynamic. The instability of this interaction has made it very difficult to visualize using traditional structure determination techniques, such as x-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy.

That's where Duke's interdisciplinary team stepped in, solving the structure using protein engineering, sophisticated NMR and software specifically designed to run on limited data.

First author Patrick Reardon spent years engineering a protein that incorporated the HIV MPER, associated with a membrane and behaved just like gp41 in the tricky intermediate step, but was stable enough to study. Reardon, then a PhD student under Spicer, is now a Wiley postdoctoral fellow at the Environmental Molecular Sciences Laboratory, a scientific facility in the Department of Energy's Pacific Northwest National Laboratory.

The result captured the shape of the three-parted MPER in its near-native state, but the protein needed to be more than structurally accurate—it had to bind the broadly neutralizing antibodies.

"One of the most important aspects of the project was ensuring that this construct interacted with the desirable antibodies, and indeed, it did so strongly," Reardon said.

The team validated the initial structure using an independent method of data analysis developed by Donald's lab, which showed alternate structures were not consistent with the data.

"The software took advantage of sparse data in a clever way that gave us confidence about the computed structure," Donald said. It used advanced geometric algorithms to determine the structure of large, symmetric, or membrane-bound proteins—varieties that are very difficult to reconstruct from NMR data.

Donald's lab has been perfecting the method for a nearly decade, and Donald said its application in this paper represents a culmination of that work, demonstrating how the two-pronged approach can illuminate the

structure of complex protein systems.

The next steps of this research have already begun. In December, Duke received a grant of up to $2.9 million from the Bill & Melinda Gates Foundation to fund the development of an HIV vaccine that will build on these findings.

More information: "Structure of an HIV-1 Neutralizing Antibody Target. A Lipid Bound gp41 Envelope Membrane Proximal Region Trimer." Patrick Reardon, Harvey Sage, et al. *Proceedings of the National Academy of Sciences*, Jan. 13, 2014. DOI: 10.1073/pnas.1309842111

Provided by Duke University