Researchers discover how stealthy HIV protein gets into cells

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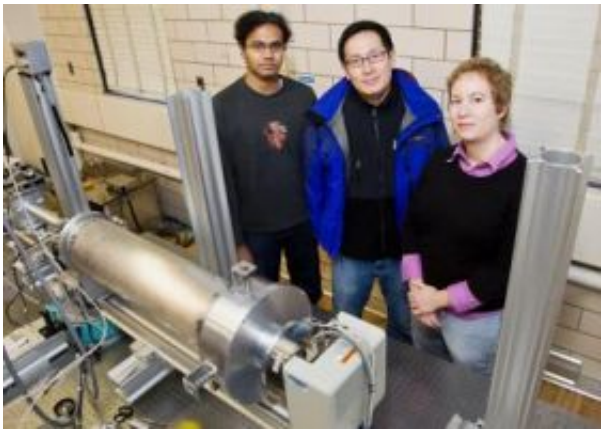

A multidisciplinary team -- graduate student Abhijit Mishra, left; materials science and engineering professor Gerard Wong; and postdoctoral researcher Vernita Gordon -- has solved the mystery of how a stealthy HIV protein gets into cells. They are standing next to a small angle X-ray spectrometer. Their findings could improve the design of therapeutic agents that cross a variety of membrane types. Credit: Photo by L. Brian Stauffer, U. of I. News Bureau

Scientists have known for more than a decade that a protein associated with the HIV virus is good at crossing cell membranes, but they didn't know how it worked. A multidisciplinary team from the University of Illinois has solved the mystery, and their findings could improve the design of therapeutic agents that cross a variety of membrane types. A paper describing their findings appears this month in *Angewandte Chemie*.

The TAT protein transduction domain of the HIV virus has some remarkable properties. First, it is a tiny part of the overall TAT protein, containing only 11 amino acids. Second, and more important, it has an uncanny knack for slipping across membranes, those lipid-rich bags that form the boundaries of cells and cellular components and are designed to keep things out.

“TAT is extremely good at getting through cell membranes,” said materials science and engineering professor Gerard Wong, who led the new study. “You can attach TAT to almost anything and it will drag it across the membrane. It can work for virtually all tissues, including the brain.”

The TAT protein’s versatility makes it desirable as a drug-delivery device. It is already being used for gene therapy. (TAT is not involved in transmitting the HIV virus; it only aids the passage of other materials across the membranes of infected cells.)

Because it has so many potential uses, scientists have long endeavored to understand the mechanism that allows the TAT protein to work. But their efforts have been stymied by some baffling observations.

Six of its 11 residues are arginine, a positively charged amino acid that gives the protein its activity.

Most membranes are composed of a double layer of neutral, water-repellent lipids on their interiors, with hydrophilic (water-loving) “head groups” on their internal and external surfaces. The head groups generally carry a mildly negative charge, Wong said. Since opposites attract, it made sense to the researchers that the positively charged TAT protein would attract the negatively charged head groups on the surface of the membranes. This attraction could deform the membrane in a way that opened up a pathway through it.

If a short, positively charged protein was all that was needed for TAT to work, the researchers thought, then any positively charged amino acid should do the trick. But when they replaced the arginine in the protein with other positively charged amino acids, it lost its function. Clearly, a positive charge was not enough to make it work.

To get a better picture of the interaction of TAT with a variety of membranes, the researchers turned to confocal microscopy and synchrotron x-ray scattering (SAXS). Although sometimes used in biological studies, SAXS is more common to the fields of physics or materials science, where the pattern of X-ray scattering can reveal how atomic and nano scale materials are structured.

The researchers found that adding the TAT protein to a membrane completely altered its SAXS spectrum, a sign that the membrane conformation had changed. After analyzing the spectrum, the researchers found that TAT had made the membranes porous.

“The TAT sequence has completely reconstructed (the membrane) and made it into something that looks a little bit like a sponge with lots of holes in it,” Wong said.

Something about the TAT protein had induced a “saddle splay curvature” in the membrane. This shape resembles a saddle (like that of a Pringles potato chip), giving the openings, or pores, a bi-directional arc like that seen inside a doughnut hole.

The newly formed pores in the membrane were 6 nanometers wide, large enough to allow fairly sizeable proteins or other molecules to slip through. The pores would also make it easier for other biological processes to bring materials through the membrane.

Further analysis showed that the arginine was interacting with the head

groups on the membrane lipids in a way that caused the membrane to buckle in two different directions, bringing on the saddle splay curvature that allowed the pores to form.

When another positively charged amino acid, lysine, was used instead of arginine, the protein bent the membrane in one direction only, forming a shape more like a closed cylinder that would not allow materials to pass through.

These findings will aid researchers hoping to enhance the properties of the TAT protein that make it a good vehicle for transporting therapeutic molecules into cells, Wong said.

Wong also is a professor of physics and of bioengineering.

Source: University of Illinois at Urbana-Champaign

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