

## Imaging study shows HIV particles assembling around its genome

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(PhysOrg.com) -- The genesis of one the planet's most lethal viruses, HIV, has been caught on tape. New imaging experiments show individual HIV genomes -- strands of RNA — docking on the inner membrane of an infected cell wall as they are ensconced by HIV structural proteins.

HIV is a wily and lethal replicator. In less than 25 years, it's killed more than 25 million people. Scientists are exploring exactly how this virus reproduces because they would like to find a way to abort the process. Now, just two years after a group at Rockefeller University was the first to witness the birth of a single <u>HIV</u> particle in real time, the same team has zoomed in for a closer look at how the virus packages its genetic material as it assembles beneath the surface of an infected cell.

Their new work, published in the *Proceedings of the National Academy of Sciences*, visualizes for the first time the very beginning of particle formation, when the viral genome is trapped and then ensconced by a structural HIV protein named Gag. "We are beginning to see in better and better detail the steps involved, the order of things that occur in this process," says Sanford M. Simon, head of the Laboratory of Cellular Biophysics.

In collaboration with Paul Bieniasz, head of the Laboratory of Retrovirology and a scientist at the Aaron Diamond AIDS Research Institute, Simon is trying to figure out how HIV assembles inside an infected cell and then buds through the cell membrane on its way to



infecting other cells. The hope is that the work will prove useful in developing treatments for the millions around the planet living with the lethal virus. The research also highlights a powerful imaging technique that Simon has been refining since 1992, which allows scientists to answer questions that in the past have been the subject of a guessing game.

The research, led by Nolwenn Jouvenet, a postdoc in the Bieniasz lab, employs total internal reflection <u>fluorescence microscopy</u> (TIR-FM), a technique very different from classical methods, which shine light through the whole cell. TIR-FM adjusts the angle of the light shined on the cell so that all of the light's energy is reflected at the cell's surface, illuminating only the events that occur within tens of nanometers of its outer membrane. This eliminates from view the background noise — the bustling whirligig of activity farther within the cell — and affords a relatively uncluttered view of what the researchers actually want to observe. "The key advance here is the ability to observe, in living cells, individual molecules of RNA that constitute the HIV genome," says Bieniasz, who is also a Howard Hughes Medical Institute investigator.

The team tagged the HIV protein Gag as well as HIV's genome with fluorescing molecules detectable by the microscope. They found that a very small number of Gag molecules, which are the crucial structural element of HIV particles, are required to tie down the otherwise footloose strand of HIV RNA. Newly formed RNA-Gag complexes move slowly on the membrane for several minutes recruiting more Gag to the complex, firmly anchoring it in place. Once the HIV genome is encapsulated by Gag, forming a new viral particle, it is ready to be released from the cell membrane to infect other cells.

The work dispels other models for HIV assembly that did not have the benefit of real-time, single-virion imaging. For example, it shows that new HIV particles are assembled at the <u>cell membrane</u>, not before they



arrive there, and are constructed around individual genomes. While the new information may help scientists working on HIV drugs and vaccines, Jouvenet, Simon and Bieniasz also hope to use the microscopy technique to study what happens after the viral particle is formed — how it buds through the membrane, and how it detaches from the host cell and becomes a free agent.

More information: Proceedings of the National Academy of Sciences <u>106(45): 19114-19119 (November 10, 2009).</u> Imaging the interaction of HIV-1 genomes and Gag during assembly of individual viral particles, Nolwenn Jouvenet, Sanford M. Simon and Paul D. Bieniasz

Provided by Rockefeller University (<u>news</u> : <u>web</u>)

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