Findings reveal how dengue virus matures, becomes infectious

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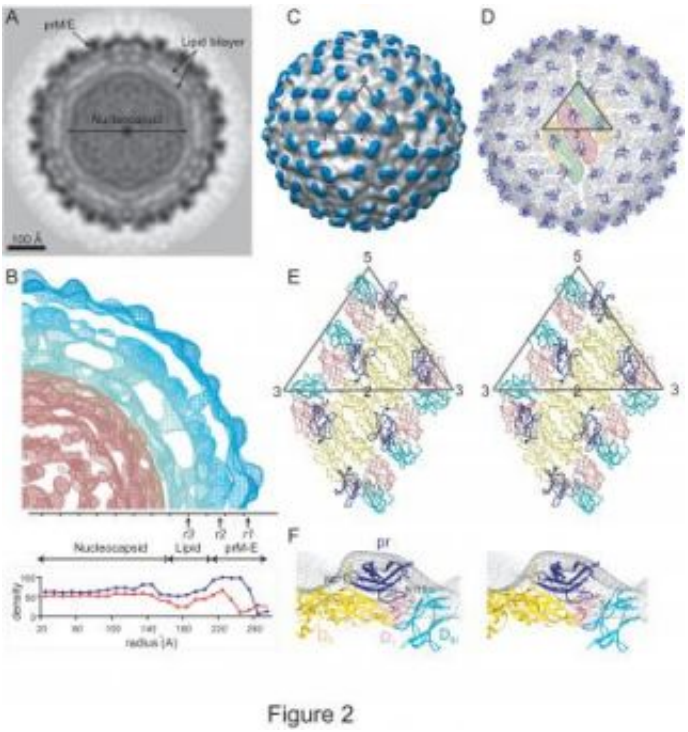

Figure 2

This composite shows an image of the dengue virus, top left, taken with cryoelectron microscopy, and, to the right of that image are reconstructions of how virus particles mature as they move through their host cells. Purdue biologists have determined why the virus undergoes structural changes as it matures in host cells and how the changes are critical for enabling the virus to infect new host cells. Other elements of the composite show structural details of the virus and a vital component made of two linked proteins called precursor membrane protein and envelope protein. Credit: Purdue University

Biologists at Purdue University have determined why dengue virus particles undergo structural changes as they mature in host cells and how the changes are critical for enabling the virus to infect new host cells.

The findings pertain to all viruses in the family of flaviviruses, which includes a number of dangerous insect-borne diseases such as dengue, West Nile, yellow fever and St. Louis encephalitis. Dengue is prevalent in Southeast Asia, Central America and South America. The virus, which is spread by mosquitoes, infects more than 50 million people annually, killing about 24,000 each year, primarily in tropical regions.

The researchers detailed critical changes that take place as the virus is assembled and moves from the inner to the outer portions of its host cell before being secreted so that it can infect other cells. Virus particles are exposed to progressively less acidic conditions as they traverse this "secretory pathway," and this changing acidity plays a vital role in the maturation of the virus.

"This is possibly the most detailed understanding of how any virus matures," said Michael Rossmann, the Hanley Distinguished Professor of Biological Sciences.

The research is a collaboration of work in two laboratories at Purdue, one operated by Rossmann and other operated by Jue Chen, an associate professor of biological sciences. They led the research with I-Mei Yu, a postdoctoral research associate working with Chen; and Long Li, a doctoral student working with Rossmann.

Findings are detailed in two back-to-back research papers appearing Friday (March 28) in the journal *Science*. The papers' co-authors include Yu, Li, Rossmann, Chen and Richard J. Kuhn, a professor and head of Purdue's Department of Biological Sciences.

Whereas the pathway for viruses entering new host cells has been studied extensively, the route for viruses moving out of their original host cells is not well-understood, Rossmann said.

"These two papers concern that route and compare the differences between both pathways," he said.

The virus moves through compartments inside the cell called the endoplasmic reticulum and the trans-Golgi network. While immature, virus particles are incapable of fusing with cell membranes, preventing them from infecting their own host cells and ensuring their maturation. Once mature, however, the virus is able to fuse to cell membranes, a trait that enables virus particles to infect new host cells, Chen said.

"There are many membranes in this trans-Golgi network, so the immature virus is always surrounded by membranes," Chen said. "In fact, the environment of the secretory pathway is very similar to what the virus encounters while it enters and infects a new host cell. So the question is, why doesn't the virus fuse to membranes on the way out""

The researchers have examined the crucial role played by the changing acidity as the immature virus travels through the compartments.

"This change in acidity was already known, but its impact on the maturation process was not known until these new findings," Rossmann said.

As a virus particle matures along the pathway through the host cell, it changes the protein structure, or "conformation," in its outer shell.

Yu mimicked the trans-Golgi network environment in test tubes, enabling the researchers to study the virus's changing structure with increasing acidity.

The surface of each virus particle contains 180 copies of a component made of two linked proteins called precursor membrane protein and envelope protein.

The precursor membrane protein prevents the immature virus from fusing with membranes by covering an attachment site in the envelope protein. During maturation, an enzyme called furin snips the connection between the two proteins, eventually exposing the envelope protein site and enabling the virus to fuse with membranes.

Yu learned, however, that the precursor membrane protein remains in place until the virus is ready to exit the original host cell. The researchers used a technique called cryoelectron microscopy to gain a more detailed view of the virus.

"So, the precursor membrane protein is retained on the virus surface even after the enzyme detaches the two proteins," Chen said. "This is a critical step because the virus is ready to mature but still is incapable of fusing with membranes until after it exits its own cell."

The researchers also determined that the environment must be acidic before the enzyme will snip the two proteins, and they examined the structure to learn specifically why the increased acidity is needed.

Li used fruit fly cells to produce large quantities of the linked proteins so that researchers could study them with a method called X-ray crystallography. Using crystallography, the researchers were able to visualize and study the combined structure of the precursor membrane and envelope proteins.

"Having a better understanding of this structure will enable us to learn why the immature form does not fuse with membranes," Rossmann said. "Ultimately, researchers might want to find ways to treat or prevent viral

infections, but in order to do that we first have to learn how viruses work, how they mature and initiate infection."

To produce the complex of the two proteins, Li first had to replace the insoluble "transmembrane region" of the protein with a soluble segment, a step essential for using the fruit fly cells to manufacture the proteins. He also had to mutate the protein to remove sites where furin normally attaches, preventing the proteins from being snipped apart.

The precursor membrane protein is about as wide as 50 nanometers, or billionths of a meter, and the envelope protein is about 3 nanometers, or nearly atomic-scale. A nanometer is about the size of 10 hydrogen atoms strung together.

Future research may focus on determining the virus's changing structure in greater detail.

Source: Purdue University