Zika virus emerged as an international public health emergency this year as outbreaks quickly spread across South and Central America, and the disease was linked to serious birth defects and Guillain-Barré syndrome. Related viruses such as West Nile and dengue sicken millions every year.

Janet Smith, Ph.D., a faculty member of the University of Michigan Life Sciences Institute, explains how she and her collaborators use the tools of structural biology to better understand how these mosquito-borne pathogens operate at the molecular level, and to find potential vulnerabilities that could be exploited by a vaccine or new antiviral therapies.
**Q:** What are flaviviruses?

**A:** Flaviviruses are a family of viruses that includes dengue virus, yellow fever, West Nile, and the one that’s been in the news a lot lately, Zika.

Together they sicken many millions of people per year, and kill tens of thousands. Zika normally causes a brief flu-like illness, but there’s a big concern about birth defects caused by the virus. The flaviviruses are close relatives, so their molecular details are very similar. However, each virus infects different cell types, so the diseases they cause are radically different.

The flaviviruses are a big public health problem because, with the exception of yellow fever, there are no effective vaccines or antiviral drugs.

In the case of dengue, for example, developing a vaccine is particularly tricky. There are four types of dengue virus worldwide, and people tend to get the fatal illness if they get sick first with one type and then are infected with a different type. This has everything to do with how the virus interacts with the immune system of the patient. Zika virus is such a new public health problem that the process of developing a vaccine is still in the early stages.

**Q:** We often hear about public health efforts to combat these viruses. How does structural biology fit into the picture?

**A:** Structural biology attacks the problem from the opposite end. It uses tools like X-ray crystallography and cryo-electron microscopy to obtain three-dimensional images of these viruses and their proteins at a resolution that’s very close to the atomic level.

Having this information allows scientists to see and better understand different aspects of how the virus functions on a molecular basis — the mechanisms by which it replicates itself and interacts with the host’s immune system. This gives us important clues about vulnerabilities that we might be able to target with a drug or vaccine.

It also allows us to see subtle but important differences between the individual flaviviruses.

**Q:** What aspects of these viruses have you focused on?

**A:** Here at the Life Sciences Institute, we’ve done a lot of work on a flavivirus protein called NS1, which is secreted by infected cells into a patient’s bloodstream.

We know that NS1 helps the virus establish an infection — and that it has two different faces; one side helps the virus make copies of itself inside of an infected cell, and the other side interacts with the immune system when it’s outside the cell in the patient’s bloodstream.

A few years ago, our group was the first to solve the 3-D structure of the NS1 protein — which had resisted scientists’ efforts to isolate it. This really opened the door for starting to figure out precisely how different parts of NS1 carry out its different functions, which can help scientists around the world work on potential methods for targeting it.

**Q:** You and your collaborators recently published a study on Zika. What did you find?

**A:** We were the first to solve the structure of the full-length Zika NS1 protein. We found that despite its similarity to NS1 proteins from other flaviviruses, there are some important differences. Zika NS1 has different electrical-charge...
properties than dengue or West Nile NS1. This information could be of immediate use in developing a more accurate blood test to determine whether a person has recently been infected with Zika virus — very important information for pregnant women or couples contemplating pregnancy. The typical antibody-based blood tests for Zika are not highly reliable because a positive result could mean that a person had been infected with a different flavivirus.

Our study also advances the basic science understanding of flaviviruses. It was the first to capture the molecular structure of flexible loops on a part of the protein known as the “wing,” which had been invisible in previous studies. This shows a new way NS1 proteins might interact with the membranes inside infected cells to help the virus replicate.

In order to make enough protein to solve a detailed 3-D structure by X-ray crystallography, we have to put DNA that codes for the protein into some type of cell — bacteria, for example — and then persuade those cells to produce the protein. Then we figure out how to separate the protein from all the other molecules in the cell in a way that doesn't disturb the protein's active, folded form.

Many proteins are not very stable. Getting it to fold up correctly can be extremely difficult — but the folded form is what determines how the protein actually works.

NS1 proteins initially presented special challenges. Flaviviruses are mosquito-borne and have a natural part of their life cycle in an insect. We were finally able to convince insect cells to make the protein. Also, the NS1 protein likes to cling to cell membranes, and that made it extremely tricky to get out. One of our team members, W. Clay Brown, spent a couple of years overcoming a series of hurdles in protein production. Another member of our team, David Akey, used a new method to measure the X-ray crystallographic data and to solve the first NS1 crystal structure.

Q: What’s next for the research?
A: We are continuing to work with virologists at Purdue University to better understand how NS1 interacts with cell membranes and with patients’ immune systems and to discover yet more functions of this unusual protein. We’re also collaborating with other researchers to use the Zika NS1 structure to develop a more accurate diagnostic test for Zika infection.

Models of the NS1 protein from different flaviviruses reveal varying electrical-charge properties, which could help develop a more accurate blood test for Zika infection.