One atomic-level snapshot at a time, the LSI’s Center for Structural Biology is partnering with researchers around campus and across the country to fight cancer, stop viruses and improve human health.

Foreground: a view of the face of the NS1 protein, which allows flaviviruses like dengue fever to spread. Background: artificial membranes coated with the NS1 protein.
The first structure ever “solved” at the University of Michigan Life Sciences Institute’s Center for Structural Biology was banana lectin, a protein found in bananas that binds to sugar molecules — its precise three-dimensional shape revealed for the first time.

“Irwin Goldstein, who’s now an emeritus professor in the Department of Biological Chemistry at the Medical School, asked us to solve the structure,” says Jeanne Stuckey, Ph.D., managing director of the CSB. “He had asked other crystallographers to do it and they had tried, but couldn’t get crystals. So I said, sure, we’re developing a center here, so we’d love to do it.”

Now, nearly 700 structures and a decade later, CSB scientists have expanded on the information gleaned from that earlier work, playing a key role in an international effort to modify banana lectin to fight viruses including influenza, hepatitis C and HIV.

In October, a U-M-led team with collaborators in Germany, Ireland, Canada, Belgium and the U.S. published findings in Cell demonstrating that a version of the banana lectin protein with a small genetic modification — a single amino acid substituted for another — allowed the protein to stop deadly viruses while preventing side effects caused by the natural version. The work to date has been in tissue cultures and animal models, but researchers are hopeful that testing in human patients could begin within a few years.

“It was the insights from that initial structure that opened the door to a potential therapeutic,” says Stuckey, who is also research associate professor of biological chemistry at the U-M Medical School and of biophysics at the College of Literature, Science, and the Arts.

The new version of banana lectin is called H84T, meaning a histidine in the 84th position on the protein chain was replaced with a threonine. This tiny change keeps sugars on the surface of immune cells from attaching to multiple spots on the lectin simultaneously and triggering inflammation — yet it still allows the protein to maintain its ability to lock on to sugars on the surface of viruses, preventing them from taking over a host’s cells.

“What we’ve done is exciting because there is potential for banana lectin to develop into a broad-spectrum antiviral agent, something that is not clinically available to physicians and patients right now,” says David Markovitz, M.D., a senior leader of the 26-member team and a professor of internal medicine at the U-M Medical School. “But it’s also exciting to have created it by engineering a lectin molecule for the first time, by understanding and then targeting the structure.”

This ability to help scientists visualize and target molecular structures using X-ray crystallography and other tools is the CSB’s raison d’être. The technological tools and scientific expertise available at the CSB have spurred collaborations with researchers across U-M, around the country and, recently, internationally, to address some of today’s most pressing health challenges — including cancer, heart disease, kidney failure and viral infections.

Finally getting to see the structure was an ‘aha moment.’
Just as the 26 letters of the English alphabet can be arranged to form more than a million words, the 20 standard amino acids found in biology can be strung together in myriad sequences to form different proteins. But knowing the sequence alone isn’t enough to understand how a protein actually works.

“Proteins function in folded forms,” says Smith, who is also a professor of biological chemistry at the U-M Medical School. “It’s what this folded form looks like, its three-dimensional shape, that determines how the protein works. That’s what gives structural biology its power.”

The atomic-level detail sought by Smith, Stuckey and their colleagues is too small to be revealed with normal light sources. Instead, they have to bounce X-rays — which have a wavelength several orders of magnitude smaller than visible light — off the planes and folds of structures they need to see. And before this can be done, a great deal of preparation is required.

In many cases, scientists at the CSB first must make the protein, which is the specialty of the center’s high-throughput protein lab. In order to obtain a sufficient quantity, they often have to put a DNA blueprint for the protein into a cell — a bacteria, fungus, animal or insect cell — and then persuade the cell to produce it. Getting an intact protein out of the cell is yet another challenge.

“The protein has to be stable, it has to be pure, and it has to be fairly abundant,” Smith says. “Figuring out how to do that can be extremely complicated — that’s where the expertise comes in.”

Once a purified protein is obtained, the researchers next have to coax it into crystallizing.

“It can take as short as 30 seconds or as long as a year. It all depends on the crystal,” Stuckey notes. “In many ways, it’s an art. There is no silver bullet that says a particular set of conditions will lead to crystals every single time.”

Some crystals can be X-rayed in-house, while others are frozen and shipped to the high-powered Advanced Photon Source synchrotron at Argonne National Laboratory near Chicago — a fairly recent development that put an end to years of driving around with samples on glass slides, fretting over every bump in the road.

Using complex mathematical models, the scientists work backwards from the diffraction pattern caused by the X-rays hitting the orderly crystal to determine the precise twists and turns of the protein’s molecular structure.

“Then you’ve arrived at your starting point,” Stuckey says. With the three-dimensional map of a structure in hand, one can learn a lot about how a protein functions or why it might be malfunctioning in certain diseases.

Looking at the shape of the sites where a protein binds to other molecules can help researchers figure out how to make a drug targeting that protein more effective. For example, the CSB contributed structural analysis to help Shaomeng Wang, Ph.D., of the U-M Comprehensive Cancer Center, and his collaborators design a better version of a cancer drug that blocks the interaction between the oncoprotein MDM2 and a tumor-suppressing protein called p53. The drug, which “achieves either durable tumor regression or complete tumor growth inhibition” in mouse models, according to findings published last year in Cancer Research, has been licensed for clinical development by Sanofi.

“As more of the early phases of drug discovery shift to the academic setting, I think that this type of work is probably going to become even more important for us,” Smith says.

Beyond the tool’s utility in drug discovery, X-ray crystallography projects at the CSB run the gamut from basic research to new approaches to environmental remediation.
One of the most important breakthroughs to come out of the CSB was a collaboration between Smith’s lab and investigators at Purdue University to solve the structure of a protein that allows dengue fever and West Nile virus to replicate and spread.

Isolating the protein, called NS1, in order to study it had been a longstanding roadblock for researchers around the world.

“Nothing similar had been solved before for us to use as a roadmap,” says W. Clay Brown, Ph.D., scientific director of the CSB’s high-throughput protein lab, who chipped away at the puzzle for more than two years before sleuthing out a combination of conditions that allowed him to obtain useable copies of the protein that folded up correctly.

“Every time we conquered one hurdle, we’d run into a new one,” he says. “Being able to run many small experiments simultaneously using high-throughput methods allowed us to see at a glance which variables made the most difference.”

The group’s findings, which were published in Science last year, are an important step toward developing a vaccine or a treatment for the tens of millions of people affected each year worldwide.

“Finally getting to see the structure was an ‘aha moment,’” Smith says. “We actually had no clue what it looked like. Now we understand which part of it helps the virus replicate, and we think we know which part of it interacts with the immune system of the infected patient.

“I get a big sense of awe when I see a new structure and can use it to understand the biology — to loop back from what we see in the 3-D image to properties that have already been determined for the molecule, and use them to craft new experiments.”

An advisory panel that examined the state of biosciences at U-M for President Mark Schlissel noted that “core” facilities like the CSB are among the university’s “significant strengths.” Core labs allow the university to make a single investment in expensive, specialized technologies that can support research activities across units and disciplines, benefiting the entire institution.

Besides the Center for Structural Biology, the LSI is also home to the Center for Chemical Genomics, a core lab that specializes in high-throughput screening.

The Center for the Discovery of New Medicines — which is supported by the LSI and several other campus units — provides seed grants to U-M researchers to fund work in both LSI centers, along with two core labs in the College of Pharmacy.

“We have concentrated skills, expertise and technology that individual labs don’t need to try to replicate,” Smith says. “Many of our clients don’t have a lot of experience with crystallography — and that’s fine. That’s what we’re here for.”

U-M Health System public relations representative Kara Gavin contributed to this story.