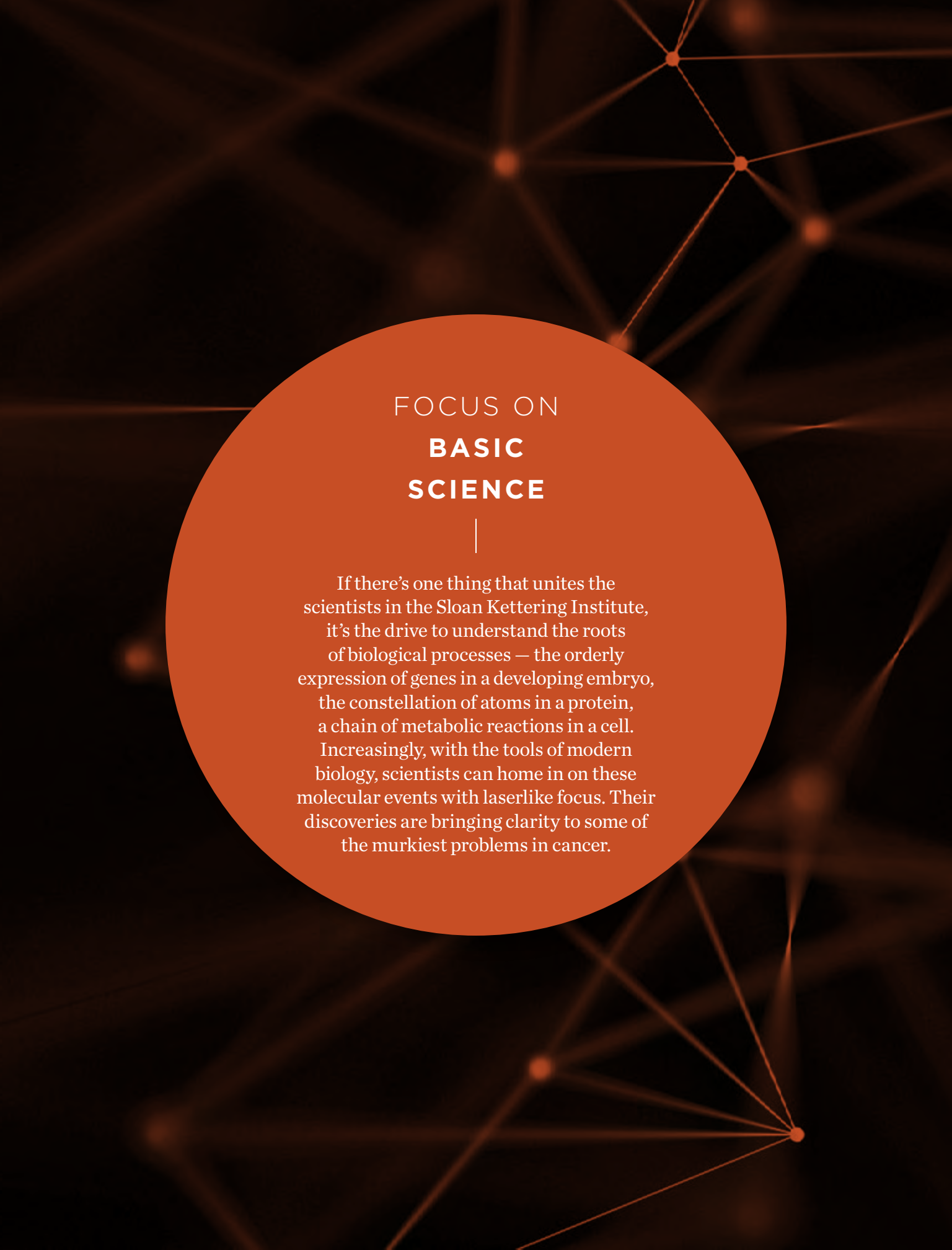


FOCUS ON **BASIC SCIENCE**

If there's one thing that unites the scientists in the Sloan Kettering Institute, it's the drive to understand the roots of biological processes — the orderly expression of genes in a developing embryo, the constellation of atoms in a protein, a chain of metabolic reactions in a cell. Increasingly, with the tools of modern biology, scientists can home in on these molecular events with laserlike focus. Their discoveries are bringing clarity to some of the murkiest problems in cancer.

Scientists are finding new ways to model disease in the lab, with the goal of determining the most effective treatments.

SEARCHING WIDE, DIVING DEEP

The genome-editing tool CRISPR has had a sizable effect on science and medicine in recent years. With these powerful molecular scissors, scientists can snip out specific pieces of DNA or make changes at precise genetic addresses. MSK researchers used CRISPR in several inventive ways this year to push cancer science forward.

Left: Ted Kastenhuber, a member of Scott Lowe's lab in the Sloan Kettering Institute, removes frozen cancer cells from liquid nitrogen. *Above*: Mr. Kastenhuber grows cancer cells in vitro. The cells are from mouse tumors that the team generated in the lab. Growing cancer cells outside the body could make it easier to test drug sensitivity more quickly and efficiently compared with studying the results in patients.

NATURE'S SWISS ARMY KNIFE: THE MANY USES OF CRISPR

UNRAVELING THE CAUSE OF A DEADLY CANCER

The rare liver cancer fibrolamellar hepatocellular carcinoma (FL-HCC) strikes fewer than 1,000 individuals a year worldwide, mostly children and young adults. There are few effective treatments besides surgery. FL-HCC is usually diagnosed late, so it is often fatal.

Because FL-HCC is so uncommon, it's hard for researchers to learn more about it, or even to conduct clinical trials of potential medicines. And without knowing more about the disease's underlying biology, they can't come up with solutions to stop it.

But what if the disease could be recreated in the lab? Could scientists use a synthetic model to test new FL-HCC drugs?

A team led by cancer biologist Scott Lowe, Chair of the Cancer Biology and Genetics Program in the Sloan Kettering Institute, decided to try just that. They used CRISPR to engineer mice with the same mutation that affects people with the disease.

It worked beautifully. "We showed that if you can reproduce that genetic event in mice, they will develop a cancer that looks very much like human FL-HCC," Dr. Lowe says. "This demonstrates that the mutant gene causes FL-HCC."

The research came about through a collaboration between Dr. Lowe's lab at MSK and Sanford Simon's lab at The Rockefeller University. Several years ago, Dr. Simon and colleagues discovered that nearly all people with FL-HCC share the same mutation in their tumors.

It was a big advance — one made even more remarkable by the fact that Dr. Simon's own daughter, Elana, suffered from FL-HCC and was centrally involved in the research effort. But still, it was only a correlation.

To find out for sure whether this mutation was responsible for driving the disease, Dr. Lowe's team, including Edward Kastenhuber, a student at the Gerstner Sloan Kettering Graduate School of Biomedical Sciences, used CRISPR to snip out the portion of the chromosome that is lost in people with FL-HCC. The remaining bits of chromosome then combined, fusing the two genes that are normally separated.

Scott Lowe creates genetically engineered mouse models to understand key cancer drivers.

> "Models give us the freedom to fail and to explore a wide variety of approaches. This way, we can accelerate the discovery of treatments that are more likely to be effective before exposing people to experimental medicines."
>
> –EDWARD KASTENHUBER

CRISPR: A BACTERIAL CUT-AND-PASTE SYSTEM

CRISPR stands for

C	R	I	S	P	R
Clustered	Regularly	Interspaced	Short	Palindromic	Repeats

These short sequences of repetitive DNA are found in bacteria and other microorganisms. Microbes use these sequences as a type of immune system against viruses.

Key to the whole operation are stretches of DNA in the CRISPR sequences that match the genetic sequence of viral DNA. When these sequences are transcribed into RNA, they bind to viral DNA and direct an enzyme — for example, one called Cas9 — to snip the DNA at the match site. This disarms the invader. Bacteria retain a record of past viral infections, much like our own immune system does, stitched into their DNA.

In 2012, scientists at the University of California, Berkeley, and elsewhere realized they could turn this bacterial cut-and-paste system into a powerful tool for genetic engineering. Researchers can give the Cas9 enzyme a synthetic RNA as a guide, one that matches a gene or DNA sequence of interest. They can then use the CRISPR-Cas9 system to make very precise cuts and other alterations in the DNA of cells.

The team plans to use the model to test a variety of drugs to see if they can slow or stop the cancer's growth. FL-HCC could be a good target for drugs called kinase inhibitors, but scientists won't know for sure until they test them.

"Models give us the freedom to fail and to explore a wide variety of approaches," Mr. Kastenhuber says. "This way, we can accelerate the discovery of treatments that are more likely to be effective before exposing people to experimental medicines." The findings were published in the *Proceedings of the National Academy of Science*s in November 2017.

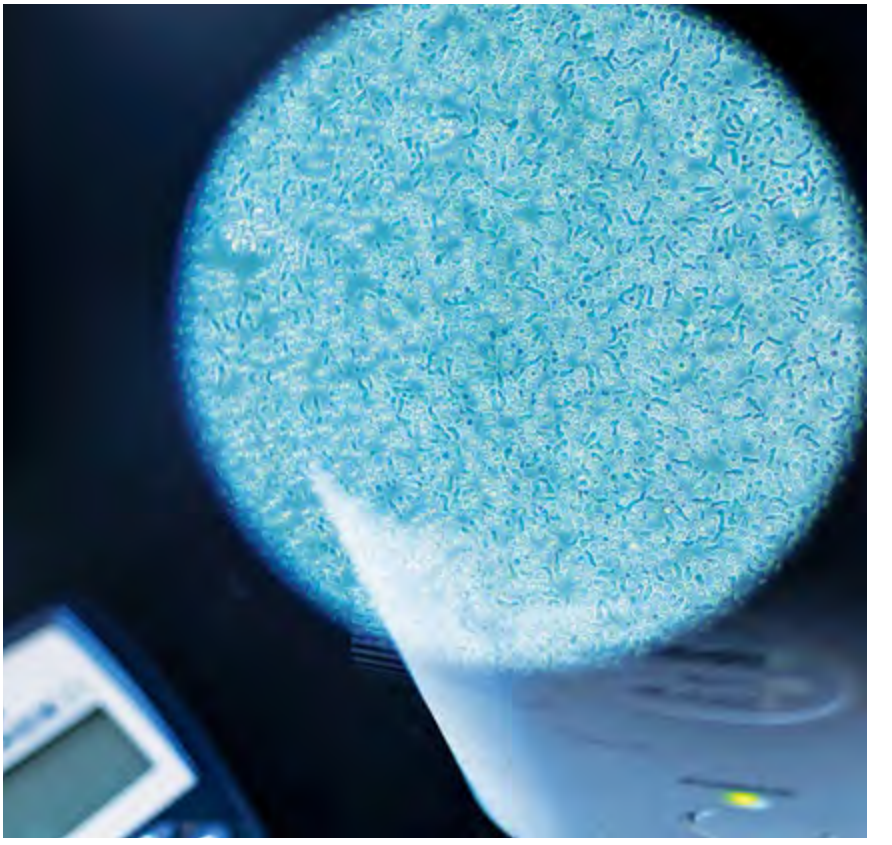
BUILDING A BETTER CAR

Chimeric antigen receptor (CAR) T cells are a powerful tool to treat certain blood cancers. (Read more about CAR T therapy on page 8.) But the way they are made is somewhat inefficient. The current process involves removing immune cells from patients and using modified viruses to deliver the CAR gene into those cells. But this method inserts the gene randomly at multiple spots in the genome. This scattershot approach isn't as efficient as it could be, and could actually cause problems by disrupting the function of important genes.

This past year, researchers in SKI used CRISPR to build a better CAR T model.

continued ▸

Below: Activated T cells, shown under a microscope, are prepared and ready for gene editing. *Bottom*: Justin Eyquem (*left*) and Jorge Mansilla-Soto are postdoctoral fellows in Michel Sadelain's lab in the Sloan Kettering Institute.



Postdoctoral fellows Justin Eyquem and Jorge Mansilla-Soto, working in the lab of Michel Sadelain, showed that if they used CRISPR to place the CAR at a very specific genome location called the TRAC locus, the cells were not only more homogeneous, they were also more effective. The approach paves the way for more reliable CAR therapies and even off-the-shelf versions that would not need to be made from a person's own cells. They published their findings in the journal *Nature* in February 2017.

"The method we developed will likely transform a costly and variable T cell–manufacturing process into a more uniform, universal, and safer one," Dr. Eyquem says.

Also remarkable is the speed at which they were able to accomplish this feat. "We started to work on this project less than three years ago, and we expect to bring it to the clinic by early 2019," Dr. Eyquem says.

KNOW THY GENOME

As precise and powerful as CRISPR can be, it's only as good as one's knowledge of the genome being cut. CRISPR can aim for multiple targets. But if it does, then instead of making precise snips in the exact spot in the genome you're aiming for, you might slice it too much.

In May 2017, researchers at SKI presented a way to solve this problem. The team — which included postdoctoral fellows Joana Vidigal and Yuri Pritykin, graduate student Alexendar Perez, and SKI faculty members Christina Leslie and Andrea Ventura — built computer software to help scientists design more-effective guide RNAs.

CRISPR uses guide RNAs to find specific regions of the genome, where it then makes a cut. The new software, called GuideScan, allows researchers to identify guide RNAs that have one or only a few possible matches in the genome, reducing the potential for too many cuts. GuideScan is freely accessible to researchers everywhere, to help enable even more skillful use of the CRISPR technology.

"Pretty much anything you can imagine, you can do," Dr. Vidigal says. "And now you can do it precisely." ■

PLUGGING A HOLE IN METASTASIS

STOPPING CANCER'S SPREAD

Cancer metastasis — which occurs when cells break off from a tumor, spread through the bloodstream or lymph vessels, and take root in another part of the body — causes the overwhelming majority of cancer-related deaths. In fact, it's widely estimated that 90 percent of cancer deaths are due to metastatic disease rather than the original tumor.

Many primary tumors can be wiped out with surgery, chemotherapy, and other treatments. Once cancer migrates, however, it becomes much harder to stop. This is especially true when it spreads to vital organs, such as the lungs, liver, or brain.

One of the most challenging types of metastatic cancer attacks the fluid and tissues that surround the brain and spine. If people develop this condition, called leptomeningial metastasis (LM), they usually die within weeks or months.

MSK cancer biologist Joan Massagué, Director of SKI and Executive Director of the Alan and Sandra Gerry Metastasis and Tumor Ecosystems Center, has been studying the intricacies of cancer metastasis for nearly two decades. Much of Dr. Massagué's current work is focused on latent metastasis, which happens when cancerous cells left behind after treatment form new cancers years or even decades later.

Joan Massagué (*center*), with medical oncologist Karuna Ganesh (*right*) and research associate Harihar Basnet, has made groundbreaking discoveries about the genes that influence how tumors interact with their environment.

> "It's difficult for cancer cells to seed new tumors in other parts of the body. To get through all the barriers that are waiting for them when they leave the mother ship, the ones that survive have to be tougher than the average cancer cell."
>
> –JOAN MASSAGUÉ

"It's difficult for cancer cells to seed new tumors in other parts of the body," he says. "To get through all the barriers that are waiting for them when they leave the mother ship, the ones that survive have to be tougher than the average cancer cell. This explains why these cells tend to be more aggressive and harder to treat."

In March 2017, Dr. Massagué and physician-scientist Adrienne Boire published a paper in the journal *Cell* on LM. To figure out how certain cancer cells grow in spinal fluid, the researchers implanted several cell lines of breast and lung cancer in mice and monitored them to see which ones resulted in LM.

To their surprise, they discovered that all of the cell lines that colonized the spinal fluid had the same well-known protein in common. After they understood how that protein worked, Drs. Massagué and Boire looked for a way to block its function. The solution was just as surprising as the initial discovery: A compound targeting the protein — originally developed to treat asthma but ultimately ineffective against that disease — suppressed LM and slowed its progression in mice. Now the researchers are investigating the possibility of using the compound to treat people with LM.

"In the past, LM was part of the fatal end stage of cancer," Dr. Boire says. By that point, people had so many other complications that not many efforts were focused on LM. But "now that patients are living longer and we're able to treat other sites of metastasis, this is becoming a clinical problem that we need to learn how to address." ■

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>
> –ADRIENNE BOIRE

Adrienne Boire (*right*), with senior research technician Majdi Alghader, was inspired to study leptomeningial metastasis by a patient of hers who had the condition.

TOR DE FORCE: CRYO-EM TECHNOLOGY

BRINGING A KEY PROTEIN INTO FOCUS

Scientists call it the master growth regulator: a protein complex in cells that senses when they have enough nutrients and cues them to grow and divide.

When this complex, called mTOR, is triggered, cells begin making copies of key ingredients, such as membranes, DNA, and organelles. They use these extra materials when they split into daughter cells.

For years, scientists have sought to target abnormally active mTOR with drugs as a way to treat cancer. Two such drugs are approved by the US Food and Drug Administration for the treatment of some types of kidney and breast cancer.

But overall, mTOR-targeted drugs have been disappointing. That may be because mTOR is a large and complicated piece of cellular equipment. There are many interacting parts, and it may be hard to take down the whole thing with a single shot. Without a clear map of the protein's structure, it's impossible to know how it works — or how to stop it.

In December 2017, a team of scientists led by Nikola Pavletich, Chair of the Structural Biology Program, and Haijuan Yang, a senior research scientist in his lab, assembled an impressively detailed view of mTOR — the first ever — including what it looks like in action. To do so, they used an innovative technology called cryo-electron microscopy (cryo-EM), a kind of satellite imagery for the cell.

With this technique, scientists shoot beams of electrons at a purified sample of molecules that is flash frozen in chilled liquid ethane. The electrons bounce off the molecules in the sample, creating an image. Thousands of individual pictures are taken, and then a scientist uses sophisticated algorithms and high-powered computers to assemble the

Nikola Pavletich (*left*), with lab member Buren Li, a student at the Gerstner Sloan Kettering Graduate School of Biomedical Sciences, studies biological molecules at an atomic level, including the proteins that control cell growth and proliferation.

pictures into a crisp, three-dimensional image. MSK installed a cryo-electron microscope, the Titan Krios, in 2017. One of its first uses was to help scientists tackle the structure of mTOR.

"Once you solve a protein's structure — in essence, creating a three-dimensional map showing where all its atoms are — you gain a much deeper understanding of how the protein works and how its function could be manipulated with drugs," Dr. Pavletich says.

Previously, such detailed atom-by-atom pictures could only be produced with X-ray crystallography, a painstaking and time-consuming method that involves first making a crystal out of a protein and then X-raying it. But not all proteins will form crystals. That's especially true of large proteins and protein complexes with multiple moving parts, like mTOR. Cryo-EM eliminates the crystallization step, which makes determining structures much easier and faster.

The mTOR protein is actually part of a larger assembly of several interlocking protein pieces that operate together. The whole complex is called mTORC1.

The structure newly obtained by cryo-EM shows how all the pieces fit together, including how mTOR is turned on. What once would have taken years to complete took just a few months.

"Before cryo-EM, people could model bits of this protein and bits of that protein," Dr. Yang says. "Now we can put it all together." ■

> **"Before cryo-EM, people could model bits of this protein and bits of that protein. Now we can put it all together."**
>
> –HAIJUAN YANG

Below: Buren Li with Haijuan Yang and the cryo-electron microscope that arrived at MSK in 2017. *Bottom*: Structural biologist Stephen Long (*right*), with lab manager Jason Cruz, also utilizes cryo-EM technology.