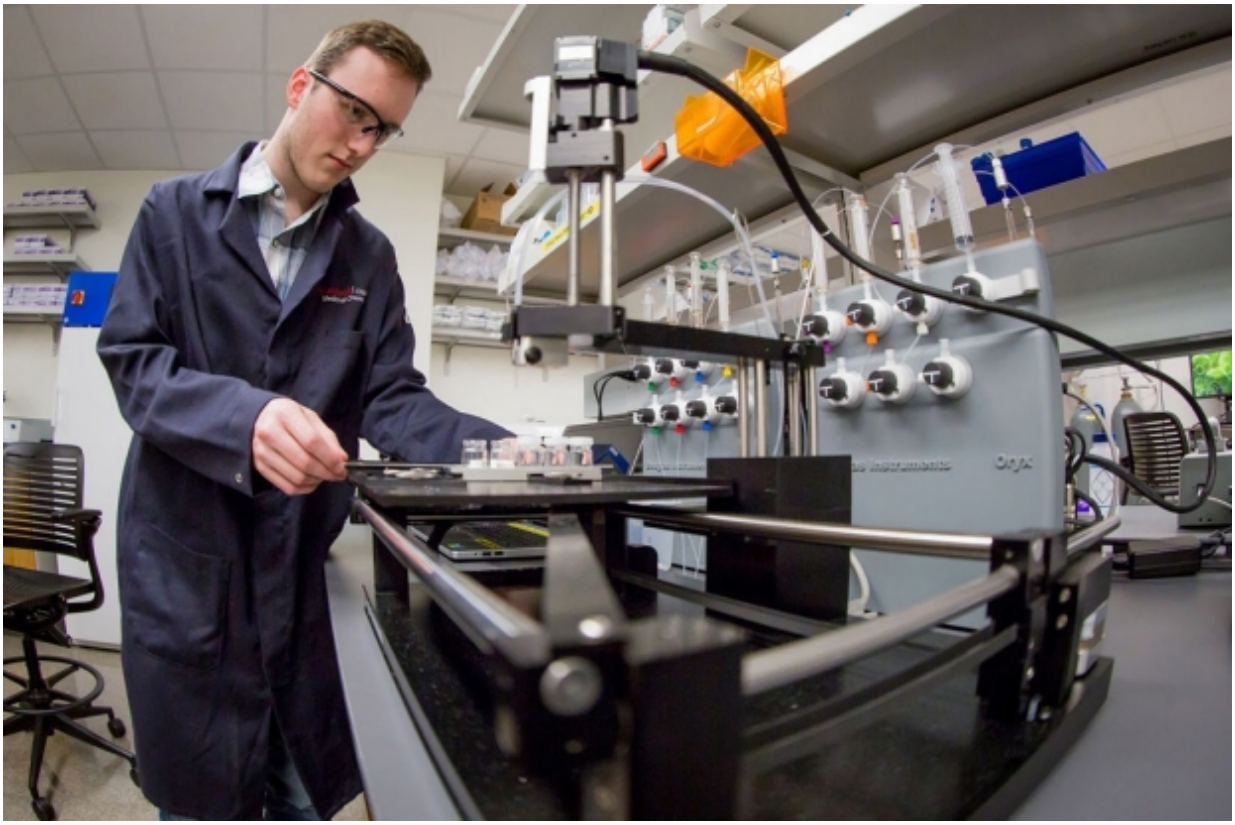
Your one-stop shop for producing, crystallizing biomolecules

April 22 2016, by Amy Adams



Undergraduate student Zachary Rosenthal loads protein samples onto the crystallization robot at Macromolecular Structure Knowledge Center, which is in the basement of the Shriram Center. Credit: Christopher Smith

A new center has been established on campus to help researchers probe the structure of biological molecules.

Housed in the basement of the Shriram Center, the Macromolecular Structure Knowledge Center contains equipment and resources for producing and crystallizing biological molecules. Among the incubators full of cells churning out molecules and crystals slowly growing in stacks of lab dishes, you'll also find Marc Deller, DPhil, who heads MSKC. He serves as a bridge between Stanford scientists hoping to understand molecular structures and the SLAC National Accelerator Laboratory, which has the SSRL Synchrotron and LCLS X-ray laser for carrying out X-ray crystallography and other structural biology techniques.

Stanford faculty may already use SLAC facilities, and many discoveries in basic structural biology and in developing drugs have been the result. But what many Stanford researchers don't have is the equipment for testing hundreds of different crystallization conditions or expertise in working with challenging molecules.

Deller has both. He has spent the past 15 years in academia and industry carrying out high-throughput protein expression, purification and crystallization, and determining molecular structures.

Marc Deller, who heads the center, inspects bacterial cultures expressing proteins targeted for crystallization trials. Credit: Christopher Smith

"It takes a bit of knowledge to know which crystallization screens to use because some are designed for a particular kind of protein, or to know how to improve the quality of the crystals for optimal data collection at SLAC," Deller said.

Crystallization resource

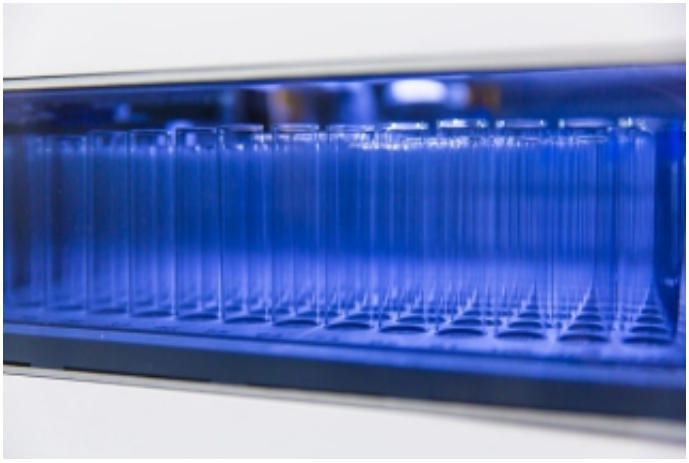
One researcher who has already found the center useful is postdoctoral scholar Lindsay Deis, PhD. She had prior experience in structural biology when she joined the lab of Peter Kim, PhD, professor of biochemistry, but Kim's lab didn't have some of the equipment Deis needed for higher-throughput crystallography.

"The MSKC opened up all these possibilities for doing structural biology," she said. "We probably could have made progress, but it would have taken a lot longer."

Deis hopes to identify some of the many structures taken on by a protein called gp41, which is located on the outside of HIV and, along with another protein called gp120, helps the virus invade cells. Many scientists have hypothesized that a drug that latches onto gp41 could prevent it from functioning and therefore stop HIV infection.

However, gp41 has proven a wily target, in part because it takes on so many shapes and is challenging to work with.

"Gp41 is a sort of unpleasant protein to work with because it is sticky," Deis said. "If you make it by itself, it likes to stick to everything, including itself, and it just becomes a big boogery mess."



The vials contain a potential drug of which Deller is helping to determine the molecular structure. Credit: Christopher Smith

She has worked with Deller and other SLAC scientists on strategies for crystallizing and determining the structure of the troublesome protein, and for modifying the usual crystallography conditions in a way that could allow her to see multiple structures.

"The staff at SLAC have been extremely accommodating and helpful, especially with some of our weird requests," she said.

In addition to providing equipment and help, the MSKC serves as a hub. "Having people to bounce ideas off of is really helpful," Deis said. "Because there are different labs coming together, you are interacting with all sorts of different people."

Creating a bridge

The idea for MSKC originated at Stanford ChEM-H, an interdisciplinary institute focused on the chemistry of human health. ChEM-H director Chaitan Khosla, PhD, professor of chemistry and of chemical

engineering, saw SLAC as a valuable and underused resource.

The institute provided seed grants to three proposed projects in 2013 to encourage more Stanford faculty to make use of SLAC facilities. But the CHEM-H members who helped design the grants quickly realized that the institute's impact would be small if it were only providing a few seed grants every other year. Instead, they reasoned, a center like MSKC could create a path to SLAC for a much larger number of faculty and projects.

MSKC is jointly supported by Stanford ChEM-H, SLAC and the School of Engineering, and supports faculty from any school.

Deller said the new facility has technology for each step of the process, starting with fermenters, incubators and bioreactors for the cell cultures that churn out molecules of interest. It also has equipment for purifying the molecules, and a crystallization robot for dispersing miniscule quantities of the purified molecule into lab plates. These plates contain a variety of different conditions—variable buffers, precipitants, different acidity levels, salts—to see which combination induces the molecule to form crystals. It's these crystals that are probed using high-intensity X-rays at SLAC probe to reveal atomic structures.

"The crystallization step would be really time-consuming without the robot," Deller said. Doing it by hand requires significantly higher quantities of the molecule, he added.

MKSC contains equipment to survey those lab dishes and scoop crystals into tanks of liquid nitrogen for transport to SLAC.

Then there's Deller, who's on hand to plan subsequent attempts if the first crystallization doesn't provide a clear structure.

"You don't usually get the structure back the first time," he said. "It is very much an iterative process. You need to have the knowledge to be able to figure out what to do next if you don't get the structure the first time."

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