Inside a high-stakes experiment in protein crystallization

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Protein-packed capsules had to be maintained at a constant temperature before experiments began.

On April 18, 2014, former astronaut and UAB Professor Lawrence DeLucas, O.D., Ph.D., stood at Cape Canaveral and watched several hundred crystallization experiments blast into orbit aboard the SpaceX Falcon 9 rocket. Then he and his team headed back to Birmingham. Another countdown had begun. When the timer stops this summer, DeLucas will have new research that could render expanded opportunities for the business and utilization of space.

Memory foam, wireless headsets, implantable insulin pumps, portable vacuum cleaners—America's space program has spun off a host of innovations that have made their way into everyday life. DeLucas, the director of UAB's Center for Biophysical Sciences and Engineering, is convinced that there is plenty of untapped potential circling the Earth in the International Space Station. He firmly believes that there are many untapped opportunities in microgravity for research and development. Now he's out to provide a comprehensive evaluation of protein crystal growth that should provide critical insight into the utilization of space.

Science among the stars

The study, titled "A Comprehensive Evaluation of Microgravity Protein Crystallization," is something of a showdown. It aims to evaluate the differences in crystallization growth and resulting protein structures: the old fashioned way, on the Earth, and in space. In addition to the academic quest for structural information on proteins, drug companies also are studying protein crystals to find new ways to block or enhance their actions in disease. If space-grown crystals can accelerate the drug-discovery process, DeLucas argues, the extra effort involved in spaceflight will be well worth it. He expects the first results to be available in June or July.

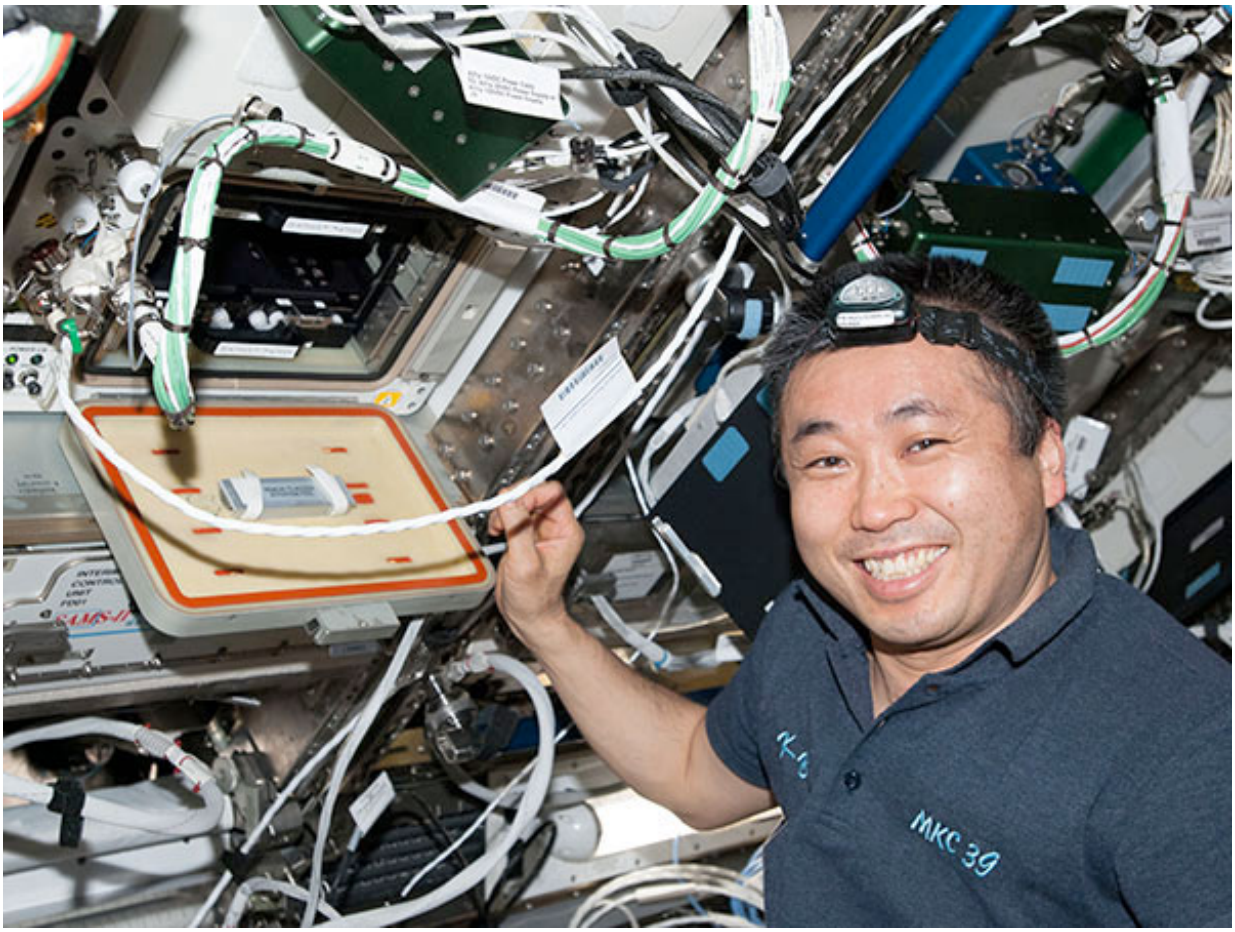
DeLucas has been working on optimizing the crystallization of proteins at UAB since the mid-1980s, when the university's renowned structural

biologist Charles Bugg, Ph.D., proposed the idea to use microgravity as a potential growth environment. In 1992, DeLucas spent 14 days aboard the space shuttle Columbia. The majority of his time was tasked with growing protein crystals (the equipment he used is now on display at the Air and Space Museum in Washington, D.C.). His dozen-plus papers, along with more than 100 other published studies, show that crystals grown in space are often larger and of higher quality than their earth-grown counterparts.

But critics have long argued that the results aren't sufficient to justify the costs associated with spaceflight. There have been successes, but also notable failures, they point out. So DeLucas proposed a comprehensive evaluation: Take 100 biologically relevant proteins, grow crystals in space and on Earth, and evaluate them using identical conditions. NASA funded the study, and researchers from across the United States, plus two international institutions, supplied batches of purified protein.

Once and for all

By the time of the April launch, 96 proteins were ready to fly, including proteins involved in several cancers, cystic fibrosis, and several other chronic and infectious diseases. For each of the proteins, CBSE scientists used different growth conditions using two different incubation temperatures.



Astronaut Koichi Wakata conducting protein crystallization experiments aboard the International Space Station.

"If the difference is negligible, you'll be able to say, 'Maybe space helps a tiny bit'; but it will be difficult to say if this is sufficient to justify the effort and cost," DeLucas said. If there is a significant difference, on the other hand, it could widen the opportunity for researchers—making the International Space Station an important tool in the drug-discovery process for certain proteins that may be difficult to crystallize on Earth.

Angst and angstroms

X-ray crystallography is one of two common ways to "solve"—that is, conclusively determine—a protein's structure. (The other is nuclear magnetic resonance spectroscopy.) Crystallography starts with crystals—comprising hundreds of thousands of protein molecules, packed together like a three-dimensional wall of bricks. By bombarding a crystal with X-rays, and using computers to analyze the resulting images, scientists can develop a picture of the protein's structure. (See graphic "Proteins in Closeup: How X-ray Crystallography Works.")

Scientists have solved the structures of some 200,000 proteins. Unfortunately, there may be as many as 1 million proteins in the body, and many of the ones involved in diseases such as diabetes and cancer don't crystallize well, or at all. The NIH is particularly interested in finding the structures of protein-protein complexes, which play a role in many disease pathways but are particularly difficult to crystallize.

Making a crystal is always a delicate process, however. The molecules need to form up in a crisply ordered fashion; the more perfectly aligned they are, the better the image. Scientists measure the limits of a crystal's resolution in angstroms; if a crystal yields a resolution of 5 angstroms, that means scientists can't distinguish anything smaller than that. (There are 254 million angstroms to the inch.) "For drug discovery, you really want something close to 2 angstroms," DeLucas said. "An improvement of a half-angstrom or more may mean the difference between being able to do the structure or not."

On Earth, gravity creates a turbulent effect that makes it harder for protein molecules to fit together in a perfect alignment. In the zero-gravity environment of space, molecules can come together in a more orderly fashion, DeLucas explains. The tradeoff is that crystal formation is slower in space, which explains some of the disappointing results from earlier space crystal studies, he adds: The 10-day flights on the space shuttle just weren't long enough to give the crystals time to grow.

That isn't a problem on the International Space Station, which remains in perpetual orbit. But keeping the proteins in space isn't the only problem; pharmaceutical companies have to get them back quickly in order to meet their strict development timeframes. "They're very interested, but they used to laugh at me when I told them I would only be able to fly every two years," DeLucas said. "Their projects are over by then. If they don't get results quickly, they have to drop it and move on to the next project."

CBSE engineers monitor conditions at the International Space Station in real time from a command center on UAB's campus.

Crystal quest

On Oct. 25, 2014, six months after DeLucas' protein samples left the planet, they splashed down in the Pacific Ocean. CBSE engineers traveled to California to collect them, returning with the precious cargo strapped into a seat on their commercial flight. Back in Birmingham, the engineers assigned code numbers to the vials from both sets of samples. Then they intermingled them to avoid any potential for bias. None of the scientists performing the analysis, including DeLucas, know which samples are from space and which are from Earth.

DeLucas' team spent the first few weeks photographing each vial under a microscope. They've devoted most days since fishing out the crystals inside, using loops that resemble tiny versions of the ones children use to blow bubbles. A delay on launch, followed by further delays, meant the crystals remained in orbit for several months longer than planned. This may have damaged several of the crystals, DeLucas notes. "However, we know that at least 50 percent of the proteins yielded crystals of sufficient size and quality—when viewed through a microscope—to enable an X-ray analysis."

Once a batch of crystals is mounted, they are sent to the high-powered synchrotron at Argonne National Laboratory outside Chicago to be analyzed using X-rays. "The synchrotron is a million times more powerful than anything we have in our lab," DeLucas said. "When you look at a crystal in a microscope, you can document its size; but you really have no idea whether it's going to be good or bad."

DeLucas' research team at Cape Canaveral before launch in April 2014.

Frozen samples are sent to Argonne by FedEx; CBSE researchers can operate the synchrotron remotely from a control room in their lab. The room-temperature samples are much more delicate. "A protein crystal is like an ordered jelly," DeLucas said. "If you touch it, it will just fall apart." These samples are ferried to Illinois in a cooler, which rides with the engineers on a commercial flight. The space and Earth crystals are imaged at the same time to make sure the conditions are exactly alike.

"That's why I titled this project 'A Comprehensive Evaluation of

Microgravity Protein Crystallization,' as we hope to once and for all provide definitive answers regarding the value of microgravity protein crystallization," DeLucas said. "I hope to demonstrate that microgravity has a positive effect on protein crystallization. That's what everyone wants clarified."

This summer, we should find out.

Provided by University of Alabama at Birmingham