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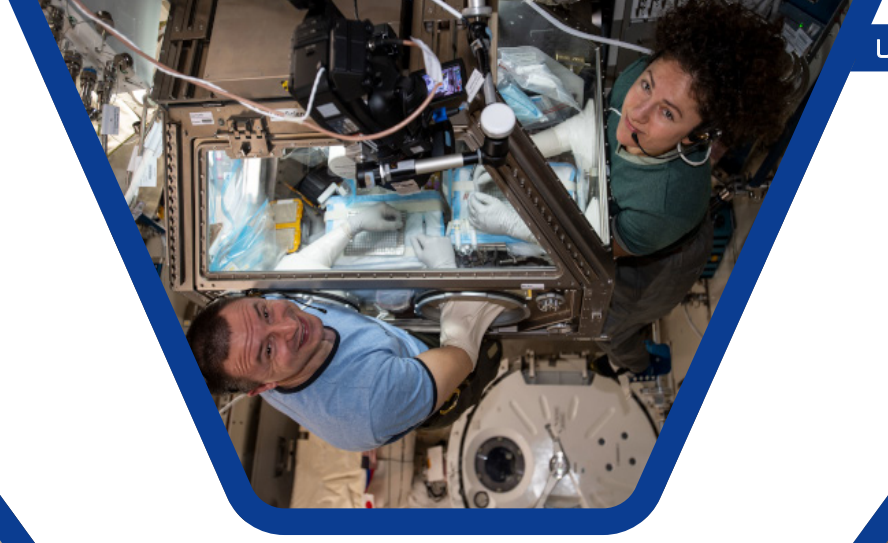

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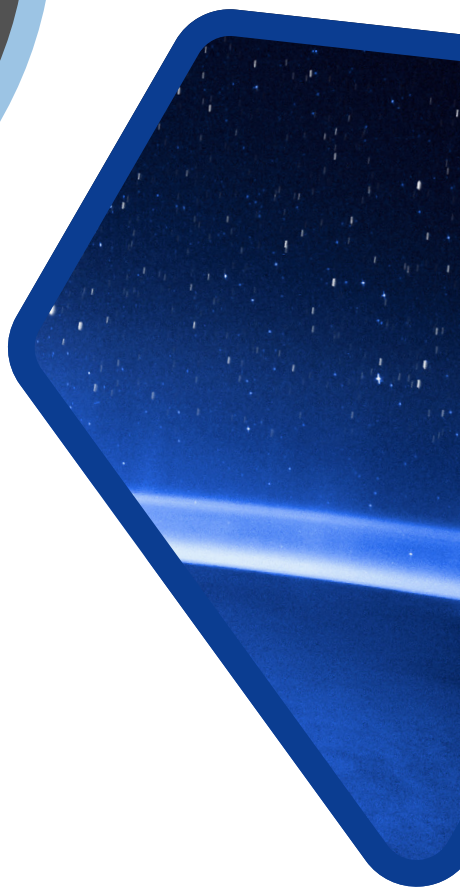

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VIEW FROM THE CUPOLA

BY AMELIA WILLIAMSON SMITH, *Upward* Managing Editor

Amelia Williamson Smith is the managing editor of Upward and the science communications manager for the ISS National Lab

Standing outside looking upward, I gaze intently into the dark night until I finally see it. It looks like a small bright dot moving across the sky, but I know it is much more than that. It is our nation's only orbiting laboratory—the International Space Station (ISS), and it enables science and technology breakthroughs not possible on Earth.

For more than 20 years, there has been a continuous human presence on the ISS. In those two decades, we have gained an incredible amount of knowledge. We have learned not only how to live and work in space but also how the unique space environment can be used to benefit life back on Earth.

After all the years of assembling the ISS, validating capabilities on station, and conducting initial studies exploring the benefits of space-based research, we have entered a new and exciting phase: a decade of results. Now, we can take all that we have learned and focus on leveraging the ISS for studies that clearly demonstrate the value of space-based research and technology development (R&D). These results are what will propel us into the future—a future of commercial space stations and a robust economy in low Earth orbit (LEO).

For the past 11 years, the Center for the Advancement of Science in Space, Inc. (CASIS) has managed the ISS National Laboratory. During this time, the ISS National Lab has sponsored more than 675 investigations that have launched to station. These projects span research areas across the board, from life and physical sciences to materials science, Earth observation, technology development, and more. Through this extensive R&D, the ISS National Lab has identified key areas of research with high potential to provide value and has concentrated efforts on advancing these areas.

As we move through this decade of results, the ISS National Lab aims to build economic value in space and support technology development that will enable the industrialization of LEO.

In this issue of *Upward*, we highlight exciting results from ISS National Lab-sponsored research in three strategic focus areas. The cover story, "Fueling the Low Earth Orbit Economy," takes readers on the journey of a small startup making big strides in creating a sustainable market in LEO. Through testing on the ISS, Orbit Fab successfully validated technology for in-space refueling, which could fundamentally change the satellite industry. The ISS serves as a valuable platform for technology demonstrations such as this that advance products and processes that will lead to positive economic impact.

The feature "Mighty Mice to the Rescue" tells the story of two researchers that came together to examine bone and muscle loss in space to improve patient care on Earth. The researchers, from the Jackson Laboratory and the University of Connecticut, used mice in microgravity to study proteins involved in muscle and bone loss that could be targeted to develop new therapeutics. Published results from fundamental science such as this provide a strong knowledge base on which future applications can be built.

In the feature, "Space Crystals and the Search for a Cancer Cure," readers discover why scientists from Frederick National Lab turned to the ISS National Lab to solve their protein crystal problem. The research team used microgravity conditions to produce improved crystals of a protein that plays a key role in several types of cancer. In-space production applications such as this demonstrate the value of space-based manufacturing and product development activities that could lead to business growth and scalable market opportunities.

In looking to the future of space-based R&D, I am excited by the endless possibilities and invaluable discoveries that await. When I see that small bright dot of the ISS moving across the night sky, I feel pride. By daring to look upward, we have opened a new frontier of science in space to improve the lives of people on Earth.

Fueling the Low Earth Orbit Economy

Orbit Fab's Gas Stations in Space

BY STEPHENIE LIVINGSTON, *Staff Writer*

A year before Orbit Fab completed tests on the International Space Station (ISS) for hardware designed to refuel spacecraft in orbit, Furphy—as the project is dubbed—was a napkin sketch, and the company was only months old.

It was 2018 and Orbit Fab was a startup with high hopes. The company had ambitions to build an in-space propellant supply chain: Gas Stations in Space™. They knew in-orbit refueling would extend the life of satellites and provide more flexibility to the industry, thus creating a more sustainable space-based economy. But first, they needed to test their hardware in space—and the ISS National Laboratory provided the perfect platform to do it.

Furphy was designed to be Orbit Fab's first in-space refueling demonstration and a chance to show the space industry that the company could back its futuristic vision with real hardware. After submitting an ISS National Lab proposal and being awarded a flight project in the summer of 2018, the company handed over hardware for a December launch on SpaceX's 16th Commercial Resupply Services mission just four and a half months later. The napkin sketch became a reality with the speed that the company has come to be known for, thanks to working with the ISS National Lab, said Orbit Fab co-founder and chief development officer Jeremy Schiel, who co-led the Furphy project.

"Currently, there's no flexibility in the industry—you build a satellite for single use, and it cannot deviate from that, primarily because it runs out of fuel," Schiel said. "So, we're fundamentally changing the entire industry."

Orbit Fab's investigation on the ISS was essential to understanding tank dynamics and pump systems in the microgravity environment where the technology must perform, an impossible task on Earth, Schiel said. Through the Furphy project, Orbit Fab successfully validated its technology. It showed the space industry that the company is a real contender to become the propellant supplier and refueling servicer in space.

Results provided Orbit Fab with proof-of-concept data to show investors, stakeholders, and potential customers, said James Bultitude, Orbit Fab's chief technology officer. "It enabled us to fly a real space mission and get real scientific results far faster than anything else we could have done on the ground," he said. Since completing the Furphy project, Orbit Fab has gone on to develop in-space refueling technology that is now commercially available.

Exploring the Business Potential of In-Orbit Refueling

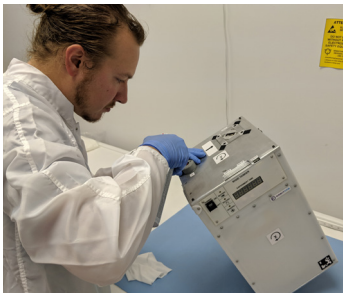
Before Orbit Fab was founded in January of 2018, Schiel and Daniel Faber, co-founder and CEO, went to various companies within the space industry and asked a simple question: What could you do with an extra tank of fuel? The response was overwhelming, according to Schiel. "We heard that many companies would see a huge increase in revenue just by having an extra kilogram of propellant in orbit," he said. "And so, after talking with several potential customers, we knew there was a business here."

At the center of Orbit Fab's mission is the idea that a robust space-based economy requires sustainable in-orbit fueling capabilities. The company's vision for the future of space is comparable to what happened on Earth when the world went from steam engines to oil and gas readily available to industry: the global shipping economy's worth went from the millions to trillions within a century.

"That was all unlocked because people had fuel to move goods and services around," Schiel said. "We're doing the same thing for space."

However, in space, fuel systems operate much differently than on the ground. On Earth, gravity forces fuel to the bottom of a car's fuel tank while a pump pushes it through the fuel pipeline to the engine. Space is a different story: liquid propellants float inside tanks and act more unpredictably. Pumps and tanks in space must be designed to deal with these difficulties.

Orbit Fab was up to the challenge and developed a pump system to move propellent between a refueling depot and spacecraft. The company also designed fuel tanks with internal baffling (panels that direct flow and help prevent sloshing) to reduce motion from liquid flow. However, the technology needed to be tested in space to ensure it worked as designed. Through the Furphy project on the ISS, Orbit Fab could test its technology and analyze pump and tank dynamics during refueling in a microgravity environment. Orbit Fab's tests on the ISS used water, but the same technology could support a wide range of propellants for spacecraft thrusters, said Bultitude. This could include common propellants like hydrazine and "green" alternatives like high test peroxide, which Orbit Fab plans to manufacture by the end of its first decade as a company.

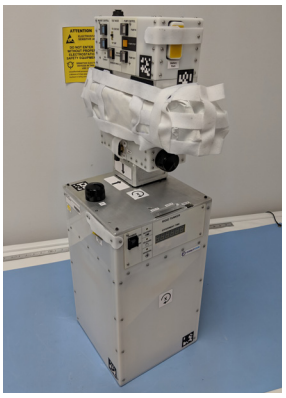


James Bultitude, Orbit Fab's chief technology officer, performs final cleaning and inspection of the rigid tanker during hardware handover to NASA before its flight to the ISS on SpaceX CRS-16.

Orbit Fab

Taking Furphy to Space

For the Furphy project, a rigid tanker the size of a 12U CubeSat (a class of nanosatellites about 20 cm by 34 cm) was filled with three gallons of water and sent to the ISS. Orbit Fab also sent an empty and compressed FlexTank™ made from silicone elastomer, which expands as it is filled in space. Because the project included a rigid tank filled with liquid, it was considered a hazardous payload, and the company worked day and night to ensure the investigation launched safely.



The FlexTank™ and rigid tank are shown here during a final connection test before launching to the ISS on SpaceX CRS-16 in 2019.

Orbit Fab

Once the payload was onboard the ISS, NASA astronaut Christina Koch tested the pump system's ability to transfer liquid between the tanker and FlexTank™ and the ability of the FlexTank™ to expand in microgravity. The test was successful, and Orbit Fab demonstrated liquid transfer between the two tanks in space.

The Furphy project also tested how tanks refueled in space are affected by propellant loads at various levels. To do this, Orbit Fab examined the dynamics of the tanker and FlexTank™ when filled with different amounts of liquid (full, half-full, a quarter-full, etc.). For each liquid level, Koch would spin the tanker, then stop it and release it to observe the tanker's behavior. Koch did the same with the FlexTank™.

Opening a New Frontier

The name "Furphy" was inspired by an Australian mobile water tank at the turn of the 20th century with the same name. The original Furphy water tank was transported via a horse-drawn cart and helped to open a new frontier in Australia—much like Orbit Fab plans to do with fuel and the space frontier.

This allowed Orbit Fab to test how well the internal baffling in the tanks reduced sloshing and residual momentum. After all the tests were complete, the water in the tanker was transferred into the space station's water supply—the first time a private company had supplied the ISS with water.

While the pumps worked well during testing with no leaks detected, and the liquid inside the tanks behaved similarly to what the company predicted, there were a few issues that Orbit Fab discovered in microgravity. The tests revealed some faults with the pump and electrical designs.

NASA astronaut Christina Koch onboard the ISS with Orbit Fab's FlexTank™ (left) and the rigid tanker (right) during testing.

NASA

"We made some design mistakes that we immediately went about correcting, which was really awesome," Bultitude said. "We had a wire that shorted on the ISS, but we managed to figure out why, and overnight, we replicated the problem on the ground and modified our engineering to remedy it."

Through successful testing on the ISS, Orbit Fab advanced the technology readiness level (TRL) of its pump system and tanks from TRL-4 to TRL-8, moving the technology closer to commercialization.

Making Big Space Industry Moves

Furphy's results continue to inform the company's engineering of future in-space tankers that will enable the refueling of satellites in orbit. With the experience and lessons gained from the project, Orbit Fab is on its way to manifesting its vision: a robust, sustainable economy in space that runs on fuel supplied by the company's fuel depots and fuel shuttles. Fuel depots are big, simple satellites full of propellants. Fuel shuttles are complex satellites that will act as servicers to pick up fuel from the depot and deliver it to the customer's spacecraft.

"So, we're not just a fuel supply chain," Schiel said. "We are a refueling service on top of the supply chain."

The company has already developed the first-ever commercially available in-space fueling port called RAFTI™ (Rapidly Attachable Fluid Transfer Interface) and the first-ever operational propellant depot in low Earth orbit (LEO), Tanker-001 Tenzing. Orbit Fab's first two GEO (geosynchronous Earth orbit) fuel shuttles will be commissioned in 2023. By providing robust refueling services in space, thereby increasing the flexibility and value of satellites, Orbit Fab is making it possible for other satellite servicing companies to perform additional maintenance tasks that further optimize satellite performance and lifespan, such as attitude control, momentum management, and relocation.

"We helped open up the space infrastructure to refueling and servicing," Schiel said. "When we started this business, there were eight satellite servicing companies. Now there are more than 100 globally."

In January 2022, Orbit Fab obtained its first customer. Astroscale, a company developing innovative in-orbit servicing solutions across all orbits, signed an agreement to refuel the company's LEXI (Life Extension In-Orbit) Servicer spacecraft; making LEXI the world's first operational commercial satellite designed to be refueled. The LEXI Servicer is expected to launch to GEO by 2026, where it will perform life extension

and other services for various customers. The RAFTI™ interface will make refueling the LEXI Servicer possible as Astroscale engineered the spacecraft's rendezvous and docking technology to allow LEXI to dock with Orbit Fab's refueling depots and shuttles.

"Think of RAFTI™ as the gas cap and interface when you refill your car," said Carolyn Belle, director of advanced systems at Astroscale. "This partnership for refueling allows us to get more use out of LEXI, just like we are helping our customers get more use out of their spacecraft."

Orbit Fab's GEO fuel shuttle will resupply Astroscale's fleet of LEXI Servicers with up to 1,000 kilograms of propellant. Schiel said Orbit Fab plans to deploy dozens of fuel tankers and shuttles in the next five to 10 years, positioning the company to refuel customer satellites in LEO, GEO, and cislunar space (the region of space from the Earth to the Moon). The plan is to be able to transfer fuel from tankers directly to operational satellites, which will be much more sustainable for several reasons, including reducing the number of fuel shuttles needed, according to Belle.

"We will not have to build new servicing spacecraft every time one runs out of fuel," Belle said. "We also will have to launch fewer additional spacecraft, which saves on emissions and resources."

And while in-orbit refueling is something that people have been interested in for a long time, no one pushed the technology and space-industry-related national and international policy forward to make it happen until Orbit Fab came along, Belle said. "Now, we have companies like Orbit Fab stepping up to say they want to do the hard work to make this possible."

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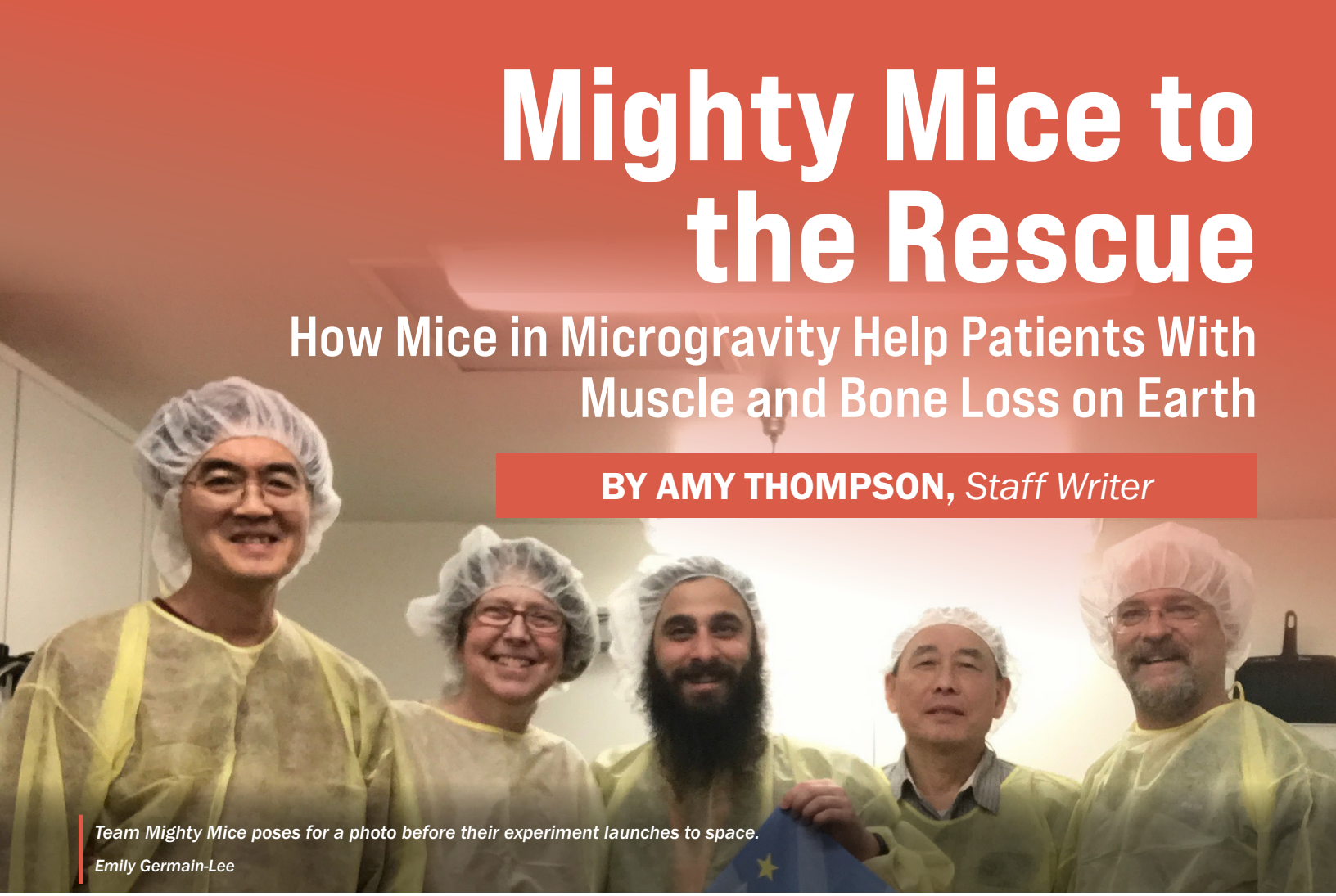
The ISS National Lab is managed by the Center for the Advancement of Science in Space, Inc. (CASIS), under Cooperative Agreement with NASA.

Mighty Mice to the Rescue

How Mice in Microgravity Help Patients With Muscle and Bone Loss on Earth

BY AMY THOMPSON, *Staff Writer*



Team Mighty Mice poses for a photo before their experiment launches to space.
Emily Germain-Lee

People usually think of bone and muscle as very different things. Bones support the body and give it shape, while muscles provide the strength to move. When now-married scientists Emily Germain-Lee and Se-Jin Lee first met in college, they were studying what they thought were separate fields: Germain-Lee focused on bone and Lee focused on muscle. However, they eventually came to realize that bone and muscle are very much tied together in fact, they are two sides of the same coin.

Over the past couple decades, we've started to see just how intertwined bone and muscle are," said Germain-Lee, a pediatric endocrinologist and professor at the University of Connecticut School of Medicine. "If the muscles aren't putting traction on the bones, then you're not going to have strong bones, and vice versa."

Because muscles and bones rely on each other to stay strong, the elderly and others with bone and muscle loss can end up in a cycle that is hard to break. "Really, anybody who has a chronic condition can become bedridden," Germain-Lee said. "It's one reason why nursing homes are packed; people break their bones, and then their muscles get weak, and they're just in bed, potentially even immobilized for the rest of their life."

To help patients such as these, Germain-Lee and Lee turned to an unexpected hero: Mighty Mice in space. The couple came together to leverage the International Space Station (ISS) National Laboratory for research that combined their areas of expertise. They conducted a rodent research investigation to see what happens when two proteins involved in muscle and bone loss—myostatin and activin A—are inhibited.

Ground studies showed that mice genetically engineered to lack myostatin developed twice the muscle mass of normal mice, hence the name "Mighty Mice." Because microgravity induces muscle and bone loss at an accelerated rate, Lee and Germain-Lee wanted to see what happened to Mighty Mice in space. "Going to space is the ultimate bed rest," said Lee, a physician and researcher at the Jackson Laboratory in Maine who provided the mice for the experiment. "So, microgravity has been instrumental in this research."

The team's findings, published in an article in the journal PNAS, revealed that inhibiting the proteins provided significant protection from muscle and bone loss in microgravity—results that could lead to new therapies for patients on Earth. "That's what really captured me," said Germain-Lee. "I saw the possibilities of what we could gain from this research, and if we had a positive result, it could really help prevent muscle and bone loss. I thought of all the people it could potentially help, and that was incredibly gripping."

Targeting Proteins Linked to Muscle and Bone Loss

In 1997, Lee had a eureka moment: he identified a gene that plays a pivotal role in regulating muscle mass. The gene triggers production of a protein called myostatin, which limits muscle growth. He discovered that if you inhibit, or block, this gene in mice, they become hypermuscular, with their muscles growing to about twice the normal size.

"That immediately suggested the possibility that if we could find a way to target this protein, it might have clinical applications to treat people with muscle loss from a wide range of conditions," Lee said. "We thought it might be a good way to develop a new therapeutic strategy to help people recover muscle mass and strength."

Lee said he then thought of astronauts in space and how, without countermeasures, they lose bone and muscle mass at an accelerated rate. It was then that he first realized he wanted to send his Mighty Mice to space. Lee petitioned NASA to conduct an experiment in space to examine how blocking the myostatin gene would affect muscle growth in mice.

A view of a wild-type mouse (left) compared with a mouse treated with the myostatin inhibitor (right) from a prior ground-based experiment.

Se-Jin Lee (2007) Quadrupling muscle mass in mice by targeting TGF-ß signaling pathways. PLOS ONE 2(8):e789. doi:10.1371/journal.pone.0000789

While he could not secure a flight in the late 1990s, Lee and others went on to discover that muscles are not the only thing affected by this gene blocking. Myostatin also affects bones.

Lee's subsequent research led to a partnership with Pfizer to develop a therapeutic that binds to myostatin to block it. Lee also found that myostatin does not work alone. Another protein, Activin A, also helps regulate muscle and bone mass.

Germain-Lee, who specializes in rare bone diseases in children, was excited about the findings. She was using mice to study a bone disorder in children, osteogenesis imperfecta (otherwise known as brittle bone disease), which affects collagen production within bones, making them incredibly weak and prone to fracture.

"A child with osteogenesis imperfecta could have 100 fractures by the time they're 10 years old, and that's no exaggeration," said Germain-Lee.

Children with this condition are often immobilized, leading to muscle atrophy, which makes physical therapy to strengthen their bones nearly impossible. Germain-Lee thought that blocking both myostatin and activin A would theoretically result in increased bone and muscle mass, which would greatly benefit children with brittle bone disease.

"We were working with mice that had osteogenesis imperfecta, and when we treated the mice to block myostatin and activin A, we saw increased bone and muscle mass," she said. "So, for me, that was incredibly exciting."

Mighty Mice in Space

Through the years, the idea of sending Mighty Mice to space never left the minds of Lee and Germain-Lee, both of whom have been fascinated with space since they were young.

Researchers Emily Germain-Lee and Se-Jin Lee watch as their research launches to space onboard a SpaceX Falcon 9 rocket.

Benjamin Charles Germain-Lee

"Everybody our age has some shared memories of space," Lee said. "We all remember watching the black and white TV and seeing Neil Armstrong and Buzz Aldrin walk on the Moon."

Germain-Lee said seeing Sally Ride fly to space when she was in medical school inspired her. "I can remember when Sally Ride was the first American woman in space, and I thought it was incredible," she said. "In those days, even as a physician, you weren't in the majority as a woman."

A few years ago, when the opportunity to send an investigation to space through the ISS National Lab came up, Germain-Lee and Lee jumped at the chance. They proposed an experiment to evaluate the effects of inhibiting myostatin and activin A on microgravity-induced muscle loss. The investigation featured 40 female mice, which included normal (or wild-type) mice, wild-type mice treated with an agent that inhibits myostatin and activin A, and genetically mutated Mighty Mice that lacked the myostatin gene.

The mice stayed onboard the orbiting laboratory for just over a month, while the researchers ran a control group consisting of the same types of mice on the ground to compare the results. While on station, the ISS crew monitored the mice, measuring their bone density, observing changes in muscle mass, and administering the inhibitor agent.

NASA astronauts Jessica Meir and Drew Morgan work with the mice in the Life Sciences Glovebox onboard the ISS.

NASA

NASA astronaut Jessica Meir uses a bone densitometer on the space station to measure each mouse's bone density throughout the experiment.

NASA

While in space, the wild-type mice lost significant bone and muscle mass, which corresponds to what we know about microgravity and the human body. However, the Mighty Mice responded differently. "The Mighty Mice had extra muscle mass as a result of having a mutation in the myostatin gene and were able to retain most, if not all, of that extra muscle during spaceflight," said Lee.

While working with the mice onboard the ISS, NASA astronaut and biologist Jessica Meir said she was surprised at how noticeable the differences were between the normal mice and Mighty Mice.

"Holding these mice, I could not only see the difference between the two groups of mice, but I could also feel how much more muscle mass the Mighty Mice had," she said.

Similarly, the wild-type mice treated with the agent that inhibits myostatin and activin A did not lose muscle and bone mass while in space. In fact, they experienced a significant increase in both. The increases in bone and muscle mass

in the treated mice in microgravity were comparable to the increases in the treated mice on the ground. This means that the spaceflight mice were protected from microgravity-induced bone and muscle loss by the treatment.

"As astronauts, we have to exercise every day to prevent bone and muscle loss," Meir said, "so having the opportunity to work on an experiment that could directly relate to a problem I faced was really rewarding."

Benefiting People on Earth and Beyond

Lee explained that the findings show that blocking the activities of myostatin and activin A works to enhance both muscle and bone, even when mice cannot bear weight, which could have significant implications for people on Earth. Targeting this pathway could be used to help people with disuse atrophy, said Lee, specifically the elderly and those who are bedridden or use a wheelchair. It could also be beneficial for those who experience muscle and bone loss due to conditions such as muscular dystrophy, osteoporosis, cancer, heart disease, sepsis, and AIDS.

"For many patients with these serious conditions, like cancer and AIDS, muscle wasting is the leading cause of death," Lee said, "Mitigating the amount of muscle mass lost would be game-changing."

The results could also have important implications for astronauts. In microgravity, nothing puts force on bones and muscles, so astronauts in space must exercise for two or more hours each day to mitigate bone and muscle loss. This research could lead to therapies that help future space travelers on long-duration missions and brief stays in low Earth orbit.

"The whole concept of this research helping astronauts really impacted me," Germain-Lee said. "Astronauts opened up a whole new world when I was a kid and seeing women like Sally Ride do these kinds of things was incredibly empowering."

Although it will be a long road to developing a treatment for patients, Germain-Lee said their space-based investigation was an important step forward. "There is still a lot of work that needs to be done," she said, "but we believe that this strategy holds promise and ultimately can improve the quality of life for many patients."

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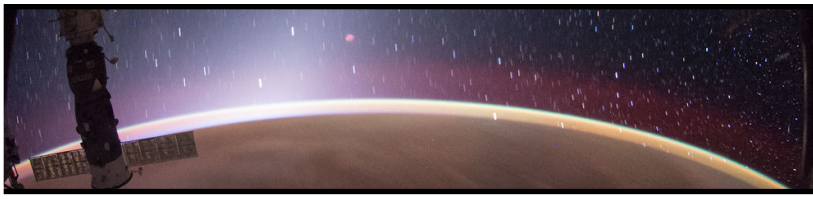

Research in space is happening now on the ISS, but how do we chart this new frontier? Our leaders at the ISS National Laboratory share insights and knowledge as we drive toward a new era of commerce and discovery in low Earth orbit.

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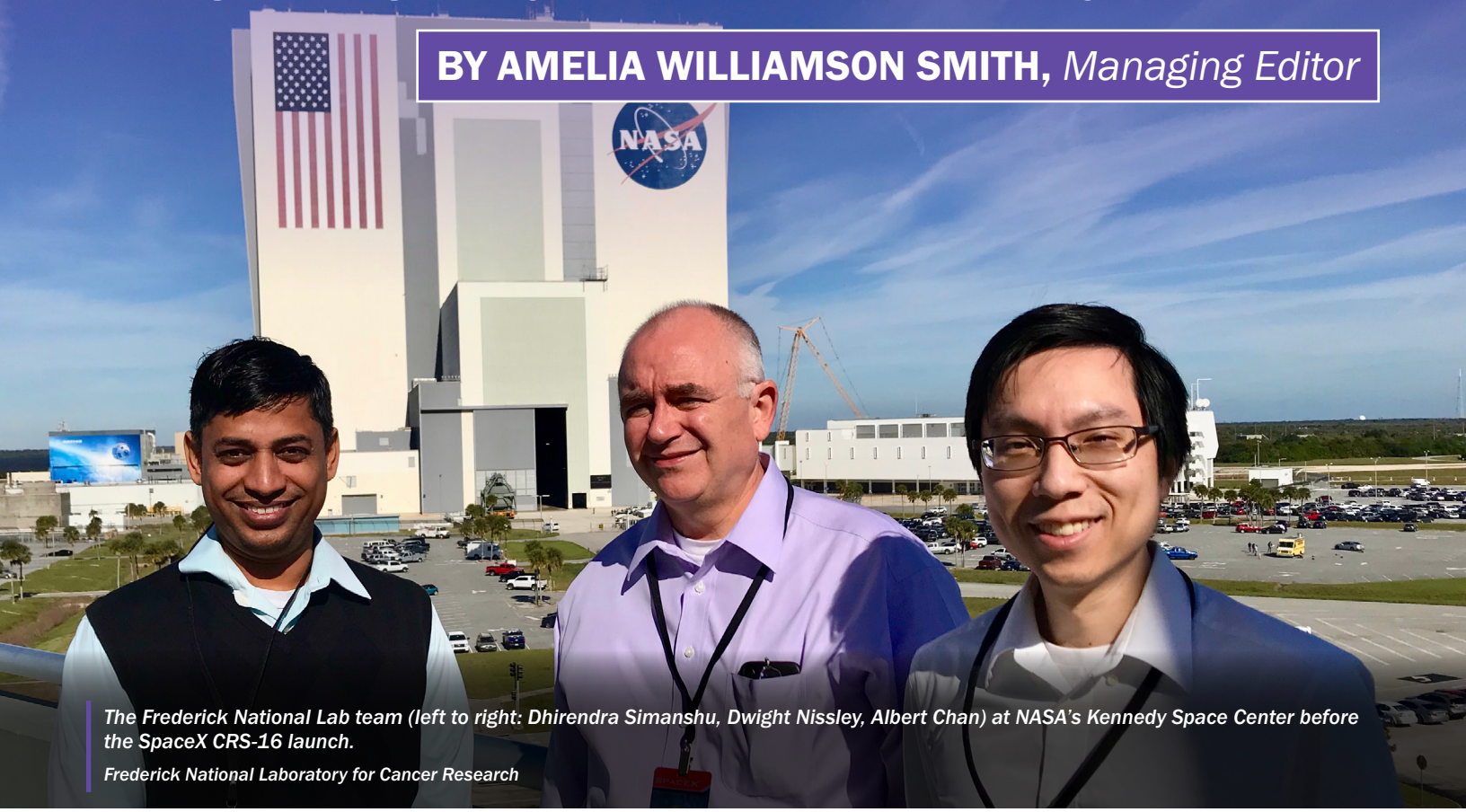
Space Crystals and the Search for a Cancer Cure

Using Microgravity to Improve Protein Crystallization

BY AMELIA WILLIAMSON SMITH, *Managing Editor*



The Frederick National Lab team (left to right: Dhirendra Simanshu, Dwight Nissley, Albert Chan) at NASA's Kennedy Space Center before the SpaceX CRS-16 launch.

Frederick National Laboratory for Cancer Research

To take a shot at a "holy grail" of cancer drug discovery, researchers from Frederick National Laboratory for Cancer Research set their sights on space. Specifically, the team leveraged the International Space Station (ISS) National Laboratory to crystallize a protein linked to several of the deadliest cancers, including pancreatic, lung, and colon cancers. Gravity-driven forces can make it difficult to grow high-quality protein crystals on Earth. However, the ISS National Lab allows researchers to do something that cannot be done on Earth—remove gravity.

KRAS is the most frequently mutated member of the RAS family of genes, which produce proteins involved in the growth and death of cells. Mutations of the KRAS gene cause about 95% of pancreatic cancer. In fact, 30% to 40% of all cancers are driven by mutations in this gene. Knowing the structure of the protein encoded by the mutated KRAS gene would allow scientists to develop drugs to block the protein's action and treat KRAS-related cancers. While scientists can crystallize proteins in their labs and use methods such as X-ray diffraction to determine the protein's molecular structure, obtaining the complete detailed structure of the KRAS protein has been challenging.

"The drug discovery effort targeting RAS-driven cancer, especially KRAS-driven cancer, hasn't been successful despite the last 20 to 30 years of effort," said Dhirendra Simanshu, a researcher at Frederick National Laboratory working on the National Cancer Institute's RAS Initiative. "So, from that perspective, this is one of the holy grails of cancer, to try to find a drug that could potentially target KRAS."

Scientists know the general shape of the KRAS protein: a ball-shaped core with a flexible tail. Using crystallography, they have determined the detailed structure of the protein's core, but it has been challenging to get a clear view of the tail because of its movement. So, the Frederick National Lab team developed a "molecular glue" to hold the tail in place during crystallization. However, when they crystallized the protein with the glue, they kept getting low-quality crystals. The data quality was so low that they could not resolve the protein structure with the glue to see if the tail held still enough to be visible. This was when they turned to space.

The Frederick National Lab team (left to right: Dhirendra Simanshu, Dwight Nissley, Albert Chan) at NASA's Kennedy Space Center before the SpaceX CRS-16 launch.

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"The problem was the internal orderliness of the crystals," said Albert Chan, who also works on the RAS Initiative at Frederick National Lab. "They were disordered, so we couldn't get very good data to resolve the structure. Microgravity can help produce better crystals that provide higher-quality data, and we were hoping to solve our crystal problem—our poor data problem—by making use of the ISS National Lab."

Getting a Look at the KRAS Protein

Proteins are complex molecules that drive cellular function to maintain our health. They are made of hundreds, or thousands, of amino acids arranged in a particular sequence, which determines a protein's 3D structure and function. Genes provide cells with instructions to produce specific proteins for different purposes. However, when a gene mutates, the instructions it sends are not quite right. When cells produce proteins that do not function correctly, it can lead to disease.

The KRAS gene encodes a protein that attaches to the cell membrane and interacts with other proteins to signal cell growth. The KRAS protein switches between an active and inactive state, which determines whether cell growth is triggered. Normally, the protein is only active in short bursts. But in the mutant form, it is always in the active state—like a car with an accelerator pedal that gets stuck. The mutant protein persistently signals cell growth, which can cause several types of cancer.

Scientists could design cancer drugs that bind to the KRAS protein and block its function, but they need the detailed structure of the full protein. The core, called the G-domain, is made of 165 amino acids that form a ball-like shape. An additional 23 amino acids extend out from the core in a highly flexible tail-like structure called the hypervariable region (HVR). The HVR plays a critical role because it attaches the KRAS protein to the cell membrane, where it interacts with other proteins to signal cell growth.

Some data suggests that when the KRAS protein is first produced, the HVR wraps back and interacts with the G-domain, forming a pocket that could be targeted for drug development, explained Dwight Nissley, director of the Cancer Research Technology Program at Frederick National Lab. A drug could be designed to bond with the protein in this initial state, locking the tail in place and preventing the protein from attaching to the cell membrane. But to do this, researchers need to see the detailed structure of the HVR and how it interacts with the G-domain. Crystallography has allowed scientists to solve the detailed structure of the G-domain, but never the HVR interacting with the G-domain because it moves too much to get a clear view.

"The HVR is quite dynamic—imagine it wiggling back and forth, so it's not caught," Nissley said. "The HVR is there in the crystal structures, but it essentially becomes lost in the noise because there's not one single conformation that is easy to see."

Developing a Molecular Glue

To try to keep the HVR at the G-domain long enough for crystallization, the Frederick National Lab team developed a molecular compound that acts like glue. "We thought that with the help of this molecular glue, it might give us a little more time for the HVR to stay attached to the G-domain so we could crystallize the full-length structure," Chan said.

In their lab, the researchers got protein crystals with the molecular glue. However, the crystal quality was not good enough to see the structure to know if the HVR was visible.

It is like a photo that is so blurred that you cannot tell if it shows what you were trying to capture.

For example, say you were trying to take a photo showing the details of individual leaves on a tree, but the image was so blurry you could not even make out the tree. You would need a higher-quality photo to see if the detailed leaves could be seen or if they were moving too much to get a clear view. In the same way, the research team needed higher-quality data to see the protein structure with the glue to find out if the HVR could be seen in detail.

"The orderliness of the crystal internally is important," Chan said. "Sometimes you can get crystals, but when you zoom in, you don't actually have perfect, orderly crystal packing in the lattice, and so you're going to get poor data quality."

Bringing Two National Labs Together

To improve the quality of the data, the team turned to the ISS National Lab. For some proteins, microgravity has been shown to produce higher-quality crystals than can be achieved on Earth. This is because, in microgravity, the movement of molecules during crystallization is slower and more ordered, resulting in a more uniform crystal.

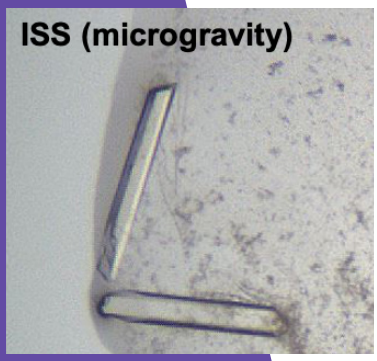
"We had heard of many cases where microgravity has helped in improving crystal quality," Simanshu said. "We cannot change gravity in any of our experiments on the ground, but doing an experiment on the ISS allowed us to evaluate that component as well, so we jumped at the opportunity." Working with another national lab was one of the reasons the team was excited about the project, Nissley said.

"National labs each have specific expertise, and the ISS National Lab is the only lab that's able to do experiments in space," he said. "It would be great to see all the national labs finding those synergies where they can help each other by working together to do something that can't be done by one lab alone."

Sending KRAS to Space

The team prepared samples of several mutant KRAS proteins with the molecular glue to send to the ISS for crystallization. The samples would remain on station for five weeks and then be returned for analysis. Watching their investigation launch was extraordinary, Simanshu said. "I'd seen launches on TV, but seeing it in real life and knowing we had something inside that capsule just gives it a very different feeling," he said.

The excitement could also be felt in Maryland, where researchers at Frederick National Lab gathered in the lab's auditorium to watch the live broadcast of the launch. "Many of us who are scientists grew up in the age of space exploration, so we have a fondness for the space program," Nissley said. "To think that something created in this building was going to fly on a spaceflight mission was really invigorating and was a powerful experience for a lot of people at the lab."



Comparison of KRAS protein crystals grown in microgravity (top) and on Earth (bottom)

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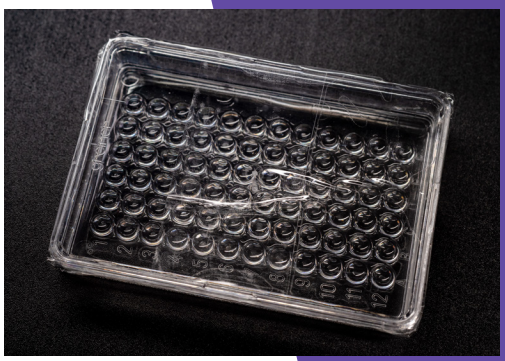

KRAS protein crystallization samples in a 96-well tray (top) and in a 72-well tray (bottom)

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European Space Agency astronaut Alexander Gerst working on the KRAS protein crystallization experiment onboard the ISS.

NASA

When the samples returned to Earth in a cargo capsule that splashed down in the Pacific Ocean, Chan was eager to bring them back to the lab for analysis. He even flew to California to retrieve the samples in person to handle the package with care. Once at the lab, Chan looked at the samples under a microscope and was excited to see several crystals. "In some of them, I noticed the crystals looked bigger with sharper edges, so they were visually better than what we had produced in our lab before," he said.

To analyze the crystals, the team worked with a third national lab—Argonne National Lab in Chicago, which houses the Advanced Photon Source that can be used for X-ray diffraction. The results showed that microgravity did solve the team's data quality problem. The ISS-grown crystals had a 50% improvement in orderliness, and the signal-to-noise was as much as five times better using the microgravity crystals versus crystals grown on the ground.

Building Incremental Success

Using the higher-quality data from microgravity crystallization, the researchers accomplished what they could not do on Earth: resolve the structure of the KRAS protein with the molecular glue. Unfortunately, however, they could only see the detailed structure of the core. The tail of the protein was still not clearly visible. Going back to the example of the tree photo—now, the tree can be seen in the picture, but the branches were moving, and the leaves are still too blurry to see details of individual leaves.

Chan said the glue may not have been strong enough, and it could be possible to develop an improved compound that keeps the HVR attached to the G-domain for longer. Additionally, for this experiment, the samples were prepared on Earth and flash frozen for launch. Once on the ISS, the samples were thawed so the crystallization process could begin. However, it would be better for the whole process to be done in microgravity and for the team to optimize the crystallization conditions for space. This could be something the team works toward in the future.

Although the full, detailed structure of the KRAS protein continues to evade researchers, and the hunt for the holy grail of cancer continues, the team's investigation was successful in a different way. The results provided important direction by allowing the researchers to see a structure they could not see in their lab.

The Frederick National Lab team carefully opening samples returned from the ISS

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It also demonstrated the potential value of microgravity crystallization for other proteins that are important drug targets but difficult to crystallize with high quality on Earth. The team's findings showed that microgravity significantly improves the quality of certain protein crystals, resulting in data that enables structure determination not possible with crystals grown on the ground.

"I wish we could say this was the greatest discovery, but I feel, like most things in science, it's incremental," Nissley said. "I always tell young people that are considering going into science: You have to be ok with 90% of what you do not working. But when you build on that knowledge and then the 10% does work—that's fantastic."

UPWARD

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