UPWARD

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GRAVITY Incarnate:

BONE HEALTH AND PHYSICAL FORCE

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VOLUME 1 ISSUE 2

CASIS™

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THE VIEW FROM THE CUPOLA

BY KENNETH A. SAVIN, ELI LILLY AND COMPANY

Kenneth A. Savin is the Advisor for Clinical Innovation at Eli Lilly and Company.

You have got to be kidding! That is all I could think two and a half years ago when I got off the phone with members of the CASIS team after being presented with the opportunity for Eli Lilly and Company to fly experiments to the International Space Station (ISS). Now, as our first experiments are launched and we have more to launch later in the year, I still have trouble believing it is really happening.

One thing I do not have trouble believing is the value that this opportunity brings to Eli Lilly and Company. When we set out to develop the experiments we would run, we had three requirements—the experiments needed to be of scientific value to Lilly, to NASA, and to people on Earth in general.

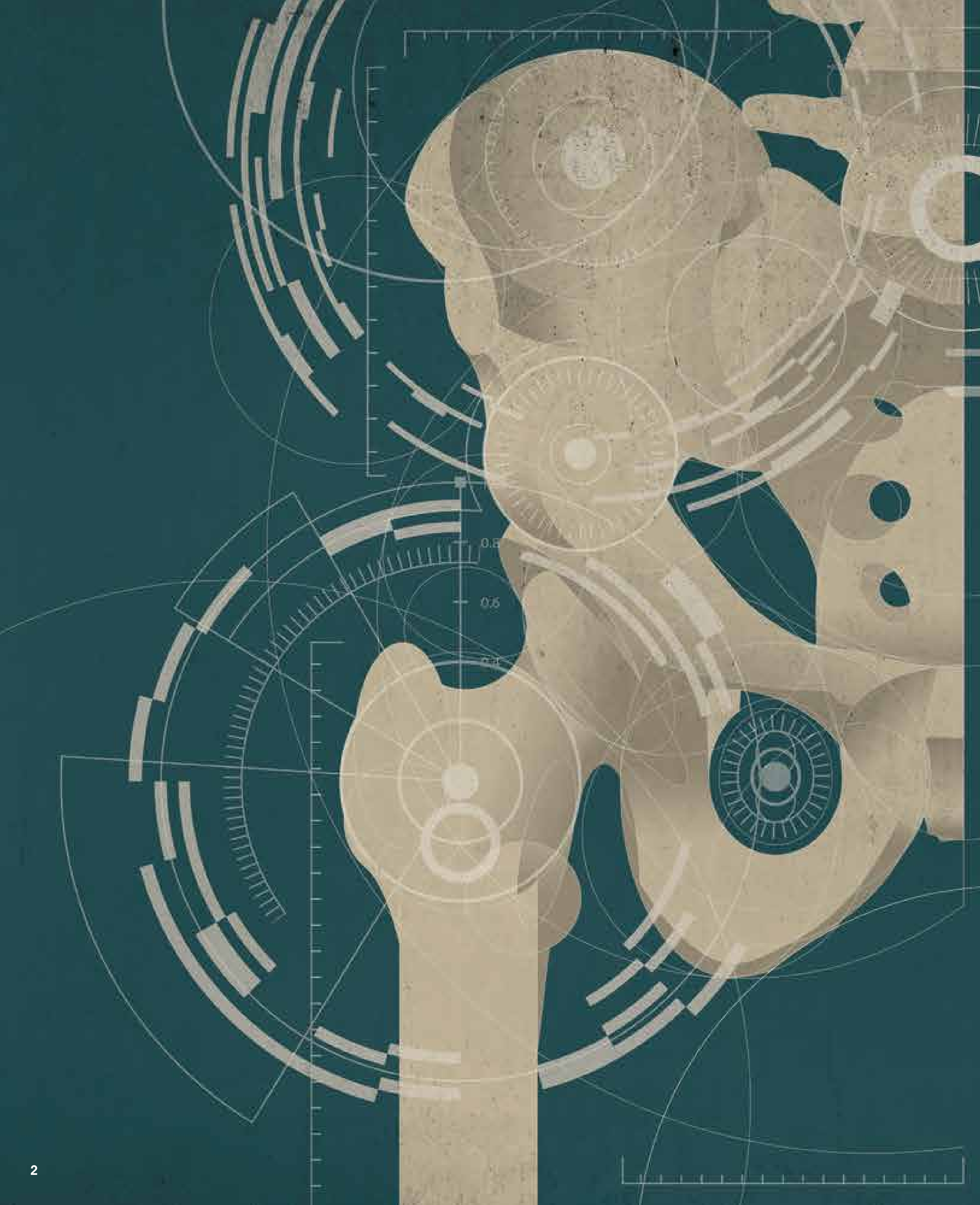
Some may ask, why spend time designing experiments for the ISS National Lab? These are experiments in which we actually want to see the results and will apply what we learn. In addition, these are all experiments that we are not able to get the results we need performing them on Earth—we need the unique microgravity environment of the ISS. What we did not understand is the kind of value the program would have for our company in other ways.

What we did not expect were the conversations around the fundamental concepts upon which our science is based. The most basic activities such as filling a vial with water have led us back to conversations around hydrogen bonding and surface tension in liquids—and that is before we actually run the experiment!

These kinds of situations and the conversations that arise lead us to reconsider what we do and what we know about our science. It causes us to reflect on our work in a way that we would not have otherwise taken the time to do. It makes us have cross-organizational conversations that lead us to new paths and other interesting possibilities. In addition, through the program, we have been introduced to new partners and new ways of thinking. These are seemingly minor activities that result in the cross-pollination of ideas and techniques, but this is how innovation happens.

Eli Lilly and Company has a history of innovation. From the early years of the company, innovation has been at the heart of our success and can be seen in the great products we have delivered over the past 140 years. Usually, when a company says it is innovative, products are pointed to as examples. But often, it is not just the "what you made," but the "how you made it" that embodies innovation and ensures that you will continue to be able to deliver great products for years to come.

The Eli Lilly and Company program to run experiments on the ISS has been of value as a catalyst for innovation at Lilly in its own way. In addition to getting us excited about being part of something special, it has pushed us to think differently and to talk about science in ways we used to as students. Now, even before we have gotten results from our experiments, it has been a positive influence on us. Once we get the results, I am sure it will send us off in other new directions toward yet unknown innovations. ■



Osteocytes comprise 90–95% of the bone cell population and reside deep within a bone's mineralized matrix, extending their branching structures across a network of cavities and channels. Their location and distribution is ideal for transmitting signals in response to external stimuli throughout the bone.

GRAVITY INCARNATE: BONE HEALTH AND PHYSICAL FORCE

BY JOHN CREECH

The human body, while often glorified in artistry for its aesthetics, is nonetheless a type of organic machinery—and like all machines, it is governed by the physical laws of nature. In a recent experiment conducted on the ISS, Boston University researchers looked beneath the superficial layers of the human form to discover the intricate beauty of bone physiology in relation to these physical forces.

The human skeleton consists of 206 individual bones. While bones may be the frequent subject of forensic postmortem analysis, it is the living tissue that gives the organ form and "plasticity," the critical ability to change and adapt.

In the ISS experiment Osteocytes and Mechano-transduction, Paola Divieti Pajevic, associate professor of molecular and cellular biology at Boston University's Goldman School of Dental Medicine, keyed in on osteocytes (the most abundant cell type in bone) to identify their role in bone health. By deciphering which parts of the osteocyte's genetic code are activated under different conditions, Divieti Pajevic and her team discovered a potential new avenue for drug development aimed at treating diseases related to bone loss.

STUDYING OSTEOPOROSIS IN MICROGRAVITY

On Earth, gravity is a constant force that imparts mechanical resistance to the body's activities. This resistance is perceived by osteocytes and translated into cellular signals that regulate the balance between tissue formation (growth) and tissue resorption (breakdown), termed bone remodeling. Osteoporosis—a condition in which bones become fragile from excessive resorption—is the most common disease related to bone remodeling, affecting more than 200 million people worldwide.

The risk for osteoporosis increases with age, especially for women with declining estrogen (a hormone that limits bone loss by inhibiting resorption). The need for new and improved therapies is critical to help prevent age-related damage to bones. Researchers are using new methods to discover factors that contribute to bone loss and may serve as alternative targets for drug development. Other approaches to developing new therapies look at reduction in physical activity—or more technically, mechanical force—as models of bone loss.

For example, bone loss in bedridden patients, such as the elderly and those with paralysis or chronic diseases, results from greatly reduced levels of regular mechanical force applied to the bones. Microgravity simulates the lack of bone and muscle loading in an accelerated manner, making the ISS National Lab an ideal environment for studying the underlying biological mechanisms related to mechanical force and bone disease. To investigate the dynamics of osteocyte signaling, gravity, mechanical force, and bone health, Divieti Pajevic took advantage of this unique research platform.

> *“We need to understand how the system works to know how it doesn’t work,” said Divieti Pajevic. “This understanding will allow us to intervene and treat bone pathology.”*

THE MACHINERY OF BONE HEALTH

In context of the body’s machinery, osteocytes are less like a linchpin locking the bone remodeling cycle statically in place and more like a transmission, able to move the cycle forward or backward. A decrease in mechanical force shifts the balance in the bone remodeling cycle in favor of resorption while an increase shifts the balance in favor of formation. This dynamic balance not only maintains bone health but also allows an organism to adjust to its environment.

One of the earliest observations regarding bone remodeling comes from Galileo Galilei, who observed that the bones of large animals (which experience increased weight-bearing mechanical force) are robustly greater in diameter relative to their length. In modern times, a similar effect is seen in professional athletes, especially those who experience unilateral mechanical force. For example, X-rays reveal the augmented growth of bone in a tennis player’s dominant hand.

"Within the bone, the cells responsible for changing bone mass according to how much external load force is applied are osteocytes,” said Divieti Pajevic. This much is known, but the mechanisms of *how* osteocytes sense and respond to mechanical force are unknown. To understand this “mechano-sensing” process and the resulting cellular signals (“mechano-transduction”) at the molecular level, Divieti Pajevic set out to perform the first-ever analysis of osteocyte gene expression in space.

This analysis was made possible through a powerful collaboration between the National Institutes of Health (NIH) and NASA: *the NIH Biomedical Research on the ISS* (BioMed-ISS) program, which specifically supported experiments in space aimed at improving health on the ground.

PERSISTENCE: THE VIRTUE OF SCIENCE

Had it not been for the dogged persistence of one graduate student (Jordan Spatz) with an unorthodox idea and the help of an international team, the Osteocytes and Mechano-transduction project may never have come to fruition. In 2010, Spatz (now a medical student at University of California, San Francisco), kicked off the initial interest in using microgravity to investigate the bone’s cellular connection with mechanical force.

Inspired by a funding opportunity from the *NIH Biomedical Research on the ISS* program, Spatz reached out to Calm Technologies, a Canadian hardware development company. The initial response was hesitance; the company’s existing hardware (originally designed for the unmanned Russian Photon spacecraft) was not certified for use on ISS.

“However, the project stood out on the strength of the science,” said Calm Technologies Vice President Chris Adamson, and this convinced the company to take on the upgrade. Iterations of the former hardware had supported three previous bone cell investigations in space. The new hardware, however, was the first to study the osteocyte cell type and the first to fly to the ISS.

“There were quite a few challenges to make the hardware ‘station-worthy,’ but it turned out to be a huge success,” said Adamson. In fact, he noted that the success of this project has generated new interest in the hardware from other investigators.

> *“BioMed-ISS was developed to facilitate NIH mission-relevant research on the ISS,” said Faye Chen of the NIH’s National Institute of Arthritis and Musculoskeletal and Skin Diseases. “The program was intended to promote biomedical research that uses the unique microgravity and radiation environment of the ISS for the benefit of human health on Earth.”*

Italian Astronaut Samantha Cristoforetti carrying out the Osteocytes and Mechano-transduction experiment onboard the ISS.

Through this unique program and later sponsorship by CASIS, the Osteocytes and Mechano-transduction experiment launched to the ISS National Lab aboard SpaceX-6 in 2015, allowing Divieti Pajevic to peer inside the osteocyte cell as it responded to the absence of mechanical force in microgravity. “There was a need to understand the mechanisms of mechano-transduction in osteocytes, the most abundant bone cells, at the cellular and molecular level,” said Chen.

HIDDEN AMONGST THE GENES

Genes and the environment are the ultimate determinants of our physical traits. Each cell in our body contains the same genetic material, but gene expression (which genes are turned on or off in any given cell) differs according to environment. GeneChip technology (see sidebar) enables analysis of which genes are turned on and off in a cell population at any given time. By comparing GeneChip data from osteocytes in spaceflight to those on Earth, changes in gene expression associated with the removal of mechanical force are highlighted.

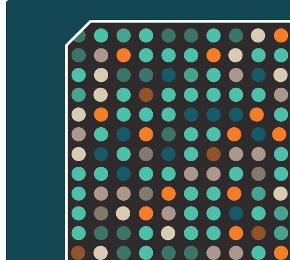
Through this approach, Divieti Pajevic discovered a surprising and exciting connection between bone cell response to mechanical force and genetic pathways not previously associated with bone disease. A significant portion of the gene expression changes in microgravity were part of the “hypoxic” pathway (a series of gene control mechanisms known for being induced by oxygen deprivation) and glycolysis (a form of glucose metabolism).

THE FUTURE OF DISCOVERY

With a new understanding of microgravity’s effect on osteocyte gene expression, Divieti Pajevic can now design experiments on Earth to more deeply understand how the pathways of hypoxia and glycolysis relate to osteocyte behavior and, more specifically, disease. Because of their association with mechano-sensing and mechano-transduction, the genes in these pathways could represent new targets for osteoporosis treatments.

“These discoveries might not otherwise have been possible through ground-based means," said Chen. Moreover, not much has been done to target these pathways in the field of osteoporosis treatment, leaving the future open for Divieti Pajevic to further elucidate the osteocyte’s genetic code. And the results gathered so far are only the beginning—analysis from the spaceflight samples is ongoing, with evaluation of protein levels and the expression of microRNAs (a recently discovered class of short RNA fragments involved in regulating gene expression) still to come.

“This was the first experiment on osteocytes in space, the first on the space station, and the first use of this hardware on the station,” said Divieti Pajevic, “so it was quite interesting to have such success and discover two new pathways that might be important for bone metabolism. We are excited about reproducing this on the ground and performing additional testing.” ■



MEASURING GENE EXPRESSION

Each spot on a GeneChip represents a specific gene. To measure which genes are being expressed (or not expressed) in space, total RNA was collected from cells in orbit and then processed on the ground to attach fluorescent labels. The fluorescently labeled RNA samples bound to their complementary gene sequence, and a laser scanner enabled a quantitative analysis of gene expression patterns. This gave the team the opportunity to see which genes were turned on or off in spaceflight compared with ground controls.

Lenore Rasmussen, Ph.D., checks pressure during the oxygen plasma treatment of titanium metal support mounts

Ras Labs-CASIS-ISS Synthetic Muscle™ experiment onboard the ISS National Lab

BRINGING MOTION TO LIFE:

MATERIALS SCIENCE RESEARCH IN SPACE

BY EMILY TOMLIN

Our hands perform dozens, if not hundreds, of tasks for us every day. They wave hello and goodbye. They open jars, doors, and soda bottles. They button our shirts, put on our makeup, and shave our beards. And often unknowingly, they gesture to add emphasis and nuance to our spoken words. For the majority of us, we take for granted the delicate, precise, and constant duties our hands perform on our command.

Now imagine that you are removed of that luxury; the hands that thanklessly performed these tasks are gone. How do you now navigate the world, and what sort of loss do you experience not only in function, but in expression and personality?

Lenore Rasmussen of Boston has devoted her career to science that enables those with lost limbs to overcome this poignant challenge. As a student, Rasmussen became intrigued by the concept of lifelike prosthetics—and for the past two decades, she has worked in the complex field of materials science and polymer chemistry to explore ways to improve prosthetics and robotics. Recently, her work has led her to a somewhat surprising testing platform: the ISS National Lab, onboard which she can study materials science in ways not possible on Earth.

THE PROMISE OF SYNTHETIC MUSCLE™

Rasmussen's passion is her quest to provide amputees with limbs that look and feel human, allowing not only robust function but also form—the artistic and nuanced movement that gives our appendages (especially our arms and hands) the ability to express emotion. Beginning with self-labeled "basement experiments" after graduate school and subsequently navigating her way through the ambitious community of biotech corporations and start-ups, Rasmussen's now 13-year-old company, Ras Labs, currently designs and tests Synthetic Muscle™, an electroactive polymer-based product that seeks to enable such humanlike prosthetics.

> *"I thought there must be a way to bridge the gap between form and function," said Rasmussen, "a different way of doing motion rather than a purely mechanical approach."*

So she explored a class of "smart" materials called electroactive polymers, which are not only much lighter than metals but also capable of converting electrical potential energy into mechanical motion without pulleys, gears, or motors.

Previous electroactive polymers have been shown to bend in response to electrical stimuli—but Rasmussen discovered specific formulations and configurations that can actually contract, in a way similar to human muscles. Synthetic Muscle™ uses these specific, patented polymers created by Ras Labs to enable biomimetic motion—lifelike dexterity, contracting, bending, and stretching—with minimal noise and heat.

In addition to contraction, the newest iterations of the Ras Labs polymers also achieve expansion, allowing truly complex motion.

Importantly, Ras Labs seeks to produce these prosthetics without high energy requirements. This feature is critical for their availability and widespread adoption—if a prosthetic can work with a small battery pack instead of a plug-in power supply, the usability for everyday citizens and functionality for military use suddenly becomes a reality instead of science fiction. Moreover, it keeps the cost down—which Rasmussen emphasizes is critical, because the places prosthetics are often most needed globally are places where people cannot afford them.

Perhaps ironically, her quest for humanlike motion has made Rasmussen's work attractive to the robotics industry, which also seeks low-power, lifelike function of artificial appendages. Contrary to Hollywood's portrayal of robots and cyborgs that are practically indistinguishable from humans, it is surprising what real-life mechanical and electronic components struggle to do—for example, turn a doorknob.

"In the first experiments testing a robot's ability to open a door, the robots fell over," said Rasmussen. "We take for granted how easy it is, but turning, oblique-type motion with our hands is very complex." The work in diagonal motion Ras Labs is exploring through the use of electroactive polymers lends itself well to solving these kinds of issues in the robotics industry.

The Ras Labs mission has thus become slightly more broad over the years—and as Rasmussen and her team expanded their goals from enabling everyday human tasks to pushing the limits of what their Synthetic Muscle™ can do for a non-human entity, they began to take their materials to the extremes.

A FLY ON THE WALL OF THE ISS

To determine whether Synthetic Muscle™ could function under stresses beyond what would be asked of a human, Rasmussen's electroactive polymers have been through a lot in the past 13 years.

In addition to being zapped by a wide range of electrical shocks, they have been chilled to a cool -271 °C, compressed by extreme G forces and pressures, and assaulted by a radioactive pellet of cesium-137 (at the U.S. Department of Energy's Princeton Plasma Physics Laboratory at Princeton University). And now, most recently, they have been Velcro-ed to the wall of the ISS for more than a year—finally returning home to Boston on June 1 after splashing down on May 11 in a Dragon capsule returning from the ISS.

The ISS testing (mainly assessing cumulative radiation exposure effects, which are present at constant low levels even in the interior of the station) will help Rasmussen determine whether robots using Synthetic Muscle™ can be sent into environments that humans either cannot enter for safety reasons or would not want to stay for long. It is obvious to see the benefits of such durability for spaceflight applications—limiting astronaut extravehicular activity would certainly be beneficial for long-term space travel—but the benefits for ground-based use are also compelling. For example, robot emergency response could save many lives in situations too dangerous for humans to act, such as inside nuclear power plants.

RAS LAB'S BUSINESS BREAKTHROUGH

Going into business was not easy for Rasmussen. She patented her discoveries and even authored a book on electroactive polymers, but her goals were always about science, discovery, and patient quality of life. In fact, she originally explored forming a nonprofit instead of a startup LLC—and she may still go that direction someday. But in 2003, she began the difficult journey of taking on partners and navigating her way through the business world as founder and chief technology officer of Ras Labs, LLC.

"In the beginning, I just wanted to see if some of my crazy ideas would work," Rasmussen said. So she started by looking at the bending of electroactive polymers, "zapping" everything she could. True to form for great discoveries, the first example of her polymers contracting instead of merely bending—giving Rasmussen the hope of biomimetic motion—happened in 2007 and was "basically a mistake in the lab," she said. "It was a very picky synthesis, so it took some work to figure out!" Luckily, the later generations of Synthetic Muscle™ are stronger, much easier to produce, and are scalable.

In this initial spaceflight experiment, various investigational additives and coatings related to Ras Labs polymers were passively exposed to the internal ISS environment, which induced changes in the materials. These changes will allow the Ras Labs team to analyze the behavior of the test materials after exposure to the specialized ISS radiation environment. While the bulk of the analyses have just begun (pH testing, material integrity, microscopy, and electroactivity), initial postflight observations and crew photography from in orbit (i.e., changes visible to the naked eye) indicate that the spaceflight results are unique from previous ground-based radiation testing. Further analysis will reveal more specifics, but Rasmussen indicated that the ISS exposure in some cases demonstrated accelerated "aging" or degradation of samples compared with ground controls.

THE CASIS CONNECTION

Ras Labs' introduction to spaceflight research came in 2013, when Rasmussen entered a competition run by MassChallenge (a global business accelerator that offers both funding and practical support). There, Rasmussen was awarded a grant through a CASIS partnership with MassChallenge—earning her an opportunity to fly her research to the ISS National Lab.

"Being on the ISS was pretty remarkable," said Rasmussen, "And it looks like we'll have some really cool, interesting results."

These results alone could help reveal unknown characteristics, including strengths and weaknesses, of the very specialized polymers Ras Labs creates and tests—which could enable design of new materials with improved features for a number of purposes (including those unrelated to spaceflight). Moreover, Rasmussen has greater plans following this initial foray into spaceflight R&D.

MINIMAL ALLOCATION FOR MAXIMUM RESULTS

The inaugural Ras Labs payload to the ISS was elegant in its simplicity. Additives and coatings for the Ras Labs custom polymers, selected for robustness based on ground-based radiation testing, were placed in tiny containers, sent up on SpaceX-6 in April 2015, and stuck to the interior wall of the ISS—using minimal power, upmass, and crew time by staying inside and requiring minimal manipulation. Two thermoluminescent dosimeters recorded radiation exposure on the ISS and on the ground duplicate controls.

THE POWER OF SPACEFLIGHT RESEARCH

Following on the heels of her promising first experiment in space, Rasmussen seeks to perform more robust spaceflight R&D that takes full advantage of the ISS environment. While passive radiation exposure is expected to yield exciting results for her team, the benefits of performing active interrogations of polymer function in microgravity onboard the ISS platform opens the door to even more revealing inquiries.

The functional absence of gravity onboard the ISS affects the structure, properties, and behavior of materials, including physical, chemical, electrical, thermal, and magnetic characteristics. These effects stem primarily from differences in fluid dynamics in space—removal of sedimentation, buoyancy-driven convection, and other phenomena that dominate fluid motion and behavior on Earth. In the absence of these phenomena, scientists are able to unmask underlying characteristics of materials and critical clues to their behavior and properties that otherwise might never be observed or understood through ground-based studies.

By testing the *function* of Synthetic Muscle™ in future materials science investigations in microgravity, Rasmussen expects to observe expansion and contraction that is more uniform, which could reveal completely new information about Ras Labs' materials. These types of studies will inform the underlying, fundamental basis upon which Ras Labs technology is built, potentially taking the company's product to whole new levels. This brings Rasmussen back to her core goals: innovative science that can improve patient quality of life.

"We need to keep up with the other new, cool developments in the industry," Rasmussen said. For example, "self-sensing," in which mechanical compression can elicit changes in the response and properties of a material. This type of feedback could enable a feature beyond only form and function: a sense of touch. Ras Labs is still working toward the seemingly simple but truly complex prosthetic hand, and Rasmussen believes that an autonomous human-like hand with actuation (including finger movement and grasping functions) is within reach for Ras Labs within five or six years.

"Our hands are close to our face; they define us as humans," said Rasmussen. "It might be a small market, but I didn't go into business to make a million and sit on a beach—I wanted to make something that people can use." ■

FINDING THE RIGHT FIT

Along with continuing to explore spaceflight R&D, Ras Labs initiated agreements with the Department of Defense and Department of Energy, and was awarded funding through the Food and Drug Administration for a project administered through the Philadelphia Pediatric Medical Device Consortium and Children's Hospital of Philadelphia that is pushing the first Ras Labs product onto the market: a shape-morphing polymer pad system for prosthetic liners and sockets.

Patient comfort is adversely affected by the common issue of poorly fitting prosthetics, which causes slippage, discomfort, and even breakdown of human tissue. Ras Labs' contracting and expanding polymers are helping to address this issue by improving and maintaining fit, allowing patients to maintain a full active lifestyle.

LAUNCHING A DREAM

EXPLORING DNA IN SPACE

BY AMELIA WILLIAMSON SMITH

Anna-Sophia Boguraev watching her experiment launch to the ISS

After years of imagining and months of preparation, New York high school student Anna-Sophia Boguraev watched in amazement as her dream left the launch pad—her *Genes in Space*™ experiment was inside the Dragon capsule on the SpaceX-8 mission headed for the ISS. Boguraev's experiment is a first step in determining whether modifications to DNA called epigenetic changes (changes that do not alter the sequence of bases that make up DNA) that are induced by spaceflight could be linked to immune system problems in astronauts living in space for an extended period of time.

"Since I was four years old, I watched rockets launch and all of the science being done in space, and I always wanted to be a part of it," Boguraev said. "Then suddenly, I was part of it—I was standing there with a rocket launching three miles away going to the ISS, and it had my experiment on it!"

Boguraev is the inaugural winner of the Genes in Space™ contest, an annual competition in which students in grades 7 through 12 compete to send their DNA experiment to the ISS. The Genes in Space™ program is supported by a partnership between Boeing, CASIS, miniPCR, Math for America, and New England Biolabs.

"We want to make sure we're doing everything possible to get the next generation of engineers and scientists engaged," said Scott Copeland, Senior Manager for Research Integration, Specialty, and Systems Engineering at Boeing. "Competitions such as Genes in Space™ are designed to foster creativity, collaboration, and critical thinking among young innovators by incorporating active learning and real-world experience."

CONNECTING EPIGENETIC CHANGES IN SPACEFLIGHT TO THE IMMUNE SYSTEM

When brainstorming ideas for experiments she could do on the ISS, Boguraev immediately thought about the immune system of astronauts.

"In order to do something groundbreaking that would be useful for humans in space, my immediate thought was to focus on the immune system," Boguraev said. "It bothered me that astronauts' immune systems do not work as well during spaceflight, because it's important for astronauts to be healthy."

Boguraev had also read that spaceflight—both environmental factors (such as microgravity and radiation) and the stress induced by spaceflight—can cause epigenetic changes in the astronauts' DNA.

Epigenetic changes are modifications made to DNA that do not involve changes to the DNA sequence. These modifications happen when chemical compounds attach to genes and affect gene expression. Boguraev knew that on Earth, some epigenetic changes can cause changes in the immune system.

This made her wonder—could the weakened immune system in astronauts be caused by epigenetic changes induced by spaceflight?

To begin to probe this question, Boguraev is examining a type of epigenetic modification called DNA methylation, which scientists have found can be affected by spaceflight.

WHAT ARE EPIGENETIC CHANGES?

The human genome is a person's complete set of DNA, the sequence of four chemical bases called adenine, cytosine, guanine, and thymine. The epigenome is the set of chemical compounds that attach to some bases in a person's DNA and help control gene expression. Epigenetic compounds do not change the DNA sequence, but change how genes are expressed by activating or silencing genes.

DNA that has been modified by epigenetic compounds is called "marked." Lifestyle and environment can cause changes in a person's epigenetic marks. Some epigenetic changes can affect the immune system or lead to disease.

DNA methylation happens when chemical compounds called methyl groups attach to cytosine bases in a strand of DNA. When regions of a gene are methylated, it prevents transcription and thus translation, so the gene is not expressed.

PHOTOCOPYING GENES IN SPACE

Boguraev's long-term goal is to develop a method for use in space to evaluate epigenetic changes affecting the human immune system. Such a process could eventually lead to the ability to monitor changes in astronauts' DNA during spaceflight and to develop countermeasures to boost their immune system when needed.

In her experiment, Boguraev used a miniPCR™ machine—a new instrument also sent to the ISS on SpaceX-8—to make billions of copies of specific genes in space for analysis back on Earth.

"It's almost anticlimactic because it doesn't look like much—it's just a strip of eight tubes with a little clear liquid in it," Boguraev said. "But I think that's the coolest part about it—while in space, billions of copies of the genes are made, but you don't see anything. The samples will come back, and it will still be eight tubes with clear liquid, but inside will be information we've never had before."

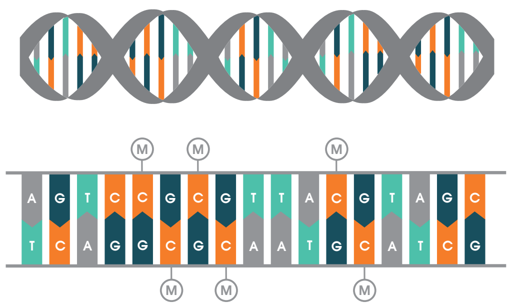
Although PCR (polymerase chain reaction) is a common chemical reaction used to amplify DNA in labs on the ground, it had never been done in space. Standard PCR machines are large, expensive pieces of equipment that can be difficult to use, said Ezequiel Alvarez-Saavedra, co-founder of miniPCR™.

HOW DOES PCR WORK?

PCR is a process first developed in 1983 that is used to make copies of specific sections of DNA targeted by small pieces of complementary DNA called primers. The process involves three steps:

1. **DENATURATION:** *In this step, the DNA sample is heated (around 94 °C) to open the two strands of DNA that make up the double helix, revealing the nucleotide bases.*
2. **ANNEALING:** *In this step, the DNA is cooled (usually between 50 °C and 60 °C), and specially designed DNA molecules called primers identify the beginning and end of the specific piece of DNA that you want to copy.*
3. **EXTENSION:** *In this step, the DNA is re-heated (around 72 °C), and an enzyme called DNA polymerase fills in the nucleotides to create a copy of the original piece of DNA.*

This cycle of heating, cooling, and re-heating is repeated about 30 times to exponentially amplify the piece of DNA, producing billions of copies.



DNA is composed of nucleotides. A nucleotide is composed of a phosphate group, a sugar group, and one of four bases—cytosine (C), guanine (G), adenine (A), or thymine (T). DNA methylation occurs when methyl groups (M) attach to cytosine bases in a strand of DNA.

The miniPCR™ machine works the same way as a standard PCR machine, but it is much more compact—small enough to hold in the palm of your hand.

> ***"We wanted to build something that could be used by anyone who wanted to do DNA analysis," Alvarez-Saavedra said. "So what we've done is create a miniPCR™ machine that is very affordable, much more portable, and is easy to use."***

DETECTING EPIGENETIC MARKS

Boguraev used the miniPCR™ machine to detect DNA methylation in genes using a technique called "methylation-specific PCR." For this technique, scientists start by treating a DNA sample with the chemical compound sodium bisulfite. This converts all un-methylated cytosine bases in the DNA into uracil while leaving the methylated cytosine bases unchanged. Uracil is one of the bases that make up RNA by replacing the thymine that is found in DNA.

Next, scientists use PCR to amplify genes in the bisulfite-treated DNA sample. Two separate PCR experiments are done simultaneously—one with primers that would amplify methylated DNA and one with primers that would amplify un-methylated DNA. In this way, scientists can determine whether the sample contained methylated and un-methylated regions of the DNA.

Boguraev's validation experiment aimed to confirm that both standard PCR and methylation-specific PCR work the same way in space that they do on the ground. To do this, she used zebrafish DNA in different states of methylation.

To test methylation-specific PCR, the zebrafish DNA was bisulfite-treated on the ground and then separated into two samples—one to be sent to the ISS and one kept on the ground. Both the spaceflight and ground samples were run through a miniPCR™ machine using the same protocol.

Boguraev's samples returned to Earth in May on the Dragon capsule, and analysis of the samples confirmed positive results. This successful proof-of-concept experiment not only enables research aimed at improving the health of astronauts in space, it also opens the door for future ISS National Lab experiments that seek to use PCR to improve human health on Earth.

Although Boguraev's samples are back on the ground, the miniPCR™ machine will stay on the ISS as part of the Boeing Science Enrichment and Engagement Kit for future ISS National Lab research and *Genes in Space*™ student projects.

SPARKING IMAGINATION

"The *Genes in Space*™ program provides a unique opportunity for students to design an experiment for a laboratory unlike any on Earth," Copeland said. This year, the *Genes in Space*™ contest received more than 300 applications submitted by more than 1,000 students (some students work in teams). The hope is that the program will continue to expand and reach more students each year.

> ***"The idea behind the*** Genes in Space™ ***program is to get students excited about science and to get them to start thinking like researchers," said Alvarez-Saavedra. "What we've seen so far is that combining biology and space ignites the imagination of students—teachers have told us they have never seen their students so excited about science, and that's exactly what our goal was."***

OTHER 2015 *GENES IN SPACE*™ COMPETITION FINALISTS

STUDENT GROUP	STATE	PROPOSED TO:
Jonathan Chang (17) and Thiago Bandeira (16)	WA	Study changes in DNA caused by cosmic radiation using a type of bacteria that can survive on the exterior of the ISS
Alyssa Huff (16)	PA	Use PCR on the ISS to test natural and unnatural genetic material in space, toward developing a method to detect extraterrestrial life
Jaclyn Shuttleworth (16), Jon Hamilton (17), and Sarah Golden (17)	MA	Explore how radiation in space affects DNA, toward designing potential shielding mechanisms
Tarun Srinivasan (17)	TX	Study changes in an astronaut's microbiome (the collection of microorganisms in a person's body) before, during, and after spaceflight

HOW DID THE *GENES IN SPACE*™ PROGRAM GET STARTED?

In 2014, Boeing and CASIS representatives came across miniPCR™ at MassChallenge, a startup accelerator and annual competition held in Boston. Representatives from miniPCR™ were showcasing their miniaturized PCR machine, which caught the interest of Boeing and CASIS.

Boeing, CASIS, and miniPCR formed a partnership, and through a mutual interest in STEM education, the Genes in Space™ program was created in early 2015.

HOW DOES THE COMPETITION WORK?

For the annual Genes in Space™ competition, students in grades 7 through 12 are invited to design a DNA experiment that could be done on the ISS. Students can submit an application individually or in teams of up to four students. Five finalists are selected from the applications received.

The finalists each work with a graduate student or post-graduate student mentor to refine their experiment idea. They also attend a workshop hosted by New England Biolabs. The finalists then each present their idea to a panel of judges at the annual ISS Research and Development (R&D) conference, and the judges select a winner. The winning team prepares its experiment for launch to the ISS the following year.

Genes in Space™ applications are due each April, finalists are announced in May, and the winner is announced in July at the ISS R&D conference. For more information about Genes in Space™, go to:

genesinspace.org

SPOT LIGHT

Reflections on Humanity BY JOHN CREECH

After 340 days on the ISS, astronaut Scott Kelly returned to Earth on March 2, 2016, bringing home a world of scientific discovery. In particular, the One-Year Mission's "Twins Study" reflects the ambitious goals of the Precision Medicine Initiative—a new national research effort that seeks to replace "one-size-fits-all" medical care with a strategy that considers individual differences in genetics, environment, and lifestyle.

NASA's Human Research Program, in collaboration with the National Space Biomedical Research Institute, is using a multi-omics approach to compare Scott with his identical twin, Mark, who remained on Earth for the duration of the mission. While Scott's return is a triumph and major milestone, the suspense remains as researchers collect postflight biological samples and continue to analyze the samples from his year in orbit.

The Twins Study consists of 10 scientific experiments, ranging from psychological to physiological, each bolstered by the twins' genomic similarity. By comparing the twins' response to stress from their respective environments, scientists can identify the connections between environment and human health. The contrasting environments thus set the stage for researchers to rigorously explore how disease and health are products of our genes (nature) and our environment (nurture).

Astronaut twins, Scott and Mark Kelly. Photo by Robert Markowitz for NASA.

For example, one experiment seeks to answer this question as it relates to the body's immune system. Changes in the twin's "immunome" as they react to a seasonal flu vaccine may reveal new possibilities for individually tailored vaccinations. The Centers for Disease Control and Prevention estimates the 2014-2015 flu vaccine was only 23% effective. While this is in part due to a poor match with circulating virus, variability in individual response also plays a role—implying the need for a precision medicine approach that is specific to each patient.

By discovering the elements of health, the Twins Study shows us what it means to be human and strives to improve biomedical understanding for both astronaut health and personalized healthcare on Earth. "We are improving our understanding of the influence of environmental stresses, like prolonged exposure to microgravity, on the dynamics of living systems," said Michael Roberts, deputy chief scientist for CASIS, "and this will lead to greater insight into the pattern and process of disease here on Earth."

OMICS: THE DATA MINING OF BIOMEDICINE

*Omics is the study of how biological elements interact on a global level (e.g., genes in the genome or proteins in the prote**ome**). The vast amount of data generated from omics studies can be analyzed with bioinformatics techniques—a combination of computer science, mathematics, statistics, and engineering.*

SPOT LIGHT

Boy Scout Team 'Ames' to Launch New Research to the ISS

BY AUSTIN JORDAN

The Boy Scouts of America offer more than 100 merit badges for youth development. Currently, there is not a merit badge for microgravity research in space—but one group of scouts is certainly making the case for a new badge. A team of young researchers in the Chicago area is designing an experiment that will study how bacteria mutate in space.

The research team is comprised of 21 members, both boys and girls, who are involved in the STEM Scouts program. STEM Scouts is a coed program of the Boy Scouts of America that offers a scouting experience focused on helping young people grow in character and skills as they explore STEM (science, technology, engineering, and mathematics) fields.

The team hypothesizes that microgravity will alter the spontaneous rate of mutation in bacteria when compared with a control experiment on the ground. Previous studies have shown that the gene expression of bacteria and other cells is altered by microgravity and that, in some cases, a bacteria's virulence (ability to infect a host and cause disease) is increased. The STEM Scouts experiment will dive deeper into this research area by conducting a modified "Ames test" in space—a test that uses bacteria to determine whether certain chemicals can cause mutations in DNA.

The STEM Scouts hope their experiment will provide further insight into cell biology and help scientists better understand how cells of all kinds—not just bacteria—evolve and respond to the environment. These insights could be helpful in understanding cell mutation as it relates to various diseases, such as cancer.

The STEM Scouts project was selected as part of the National Design Challenge competition—an ISS National Lab education program that enables student teams to propose, design, and fly to space authentic research experiments.

The project, aptly named "Our Team Ames for Space," underwent a critical design review in early June—a major milestone in the spaceflight research process. The team members presented their experimental design and updated the CASIS Operations team on their progress while receiving valuable mentoring from spaceflight research experts.

"This project marks the launch of an exciting new partnership between CASIS and the Boy Scouts of America," said Ken Shields, director of education at CASIS. "We hope that the Boy Scouts of America will become a long-term ISS National Lab education partner and help us connect even more new student communities to space science."

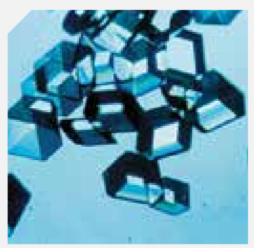
SPOT LIGHT

Innovation on the ISS to Improve Medicines on Earth

BY JOHN CREECH

Eli Lilly and Company has 140 years of pharmaceutical experience on Earth; but like imagination, innovation and discovery do not stop there. Lilly—which was among the first to mass-produce penicillin, the polio vaccine, and insulin—launched three experiments to the ISS on SpaceX-8 in April, continuing the company's tradition as a leader in research. Lilly's goal: to make better products that can improve the lives of people on Earth.

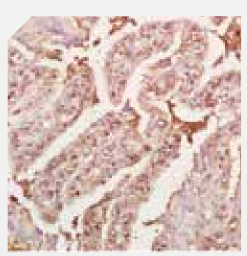
"This is an opportunity for Eli Lilly and Company to do meaningful science that would otherwise not have been possible," said Kenneth Savin, advisor of clinical innovation for Eli Lilly and Company. "At the same time, we are making a contribution that is extraordinary."



Two of Lilly's experiments involve crystallizing molecular complexes to improve structure-based drug design. In these experiments, investigators are studying small molecule inhibitors (SMIs), drugs that bind to the active site of a protein (often referred to as a "lock and key" fit) to inhibit its activity.

On Earth, sedimentation and convection (fluid movement caused by heat-induced changes in density) negatively influence the analysis of molecular interactions between a drug and its target. However, in microgravity, these forces are eliminated, allowing researchers to better crystallize SMIs bound with proteins.

The crystals recently returned to Earth for visual analysis using X-ray diffraction, a method that details molecular structure. By better understanding the molecular structure of SMIs, Lilly investigators can work toward improved drug design.



Lilly's third experiment is using rodents on the ISS to test the efficacy of a new drug (an anti-myostatin antibody) aimed at treating muscle atrophy. Myostatin is a protein that causes negative regulation of muscle growth, and Lilly's therapeutic antibody is intended to bind to myostatin and disrupt its function. If effective, this new therapy could improve the lives of a vast patient population (for example, bedridden patients such as the elderly or those with paralysis or chronic disease).

Reduced physical activity over time is a major contributor to muscle atrophy. Thus, eliminating gravity's resistive force against muscle contraction provides Lilly investigators with an accelerated model for testing the efficacy of their new drug.

With the results of these experiments pending, Eli Lilly and Company is already preparing more experiments to launch—two new studies aimed at improving drug formulation and storage. Lilly's 140 years of experience has entered a new phase: one that utilizes the unique microgravity environment of the ISS to improve pharmaceutical products that benefit lives on Earth. ■

News & Notes
FROM THE ISS NATIONAL LAB

SPACEX-8 MISSION COMPLETION

In April, aerospace manufacturer SpaceX captivated the world when the company not only successfully launched its Dragon capsule to the ISS but also landed its rocket on a floating platform in the Atlantic Ocean. While the launch (and landing) was historic, it also represented a positive step toward the two U.S.-contracted payload suppliers (SpaceX and Orbital ATK) successfully returning to flight and carrying payloads to and from the ISS. On this particular mission, new facilities were sent to the ISS along with a variety of life science payloads seeking to better understand disease.

MISSION ONE: CHICAGO

CASIS and the Boy Scouts of America hosted the Mission One: Chicago event in May to inspire the next generation of scientists and engineers, while also raising funds to promote further student engagement with the ISS. Fifteen retired astronauts joined CASIS in visiting thirteen Chicago-area schools (reaching thousands of students), with many in underserved communities. After visiting the schools, the astronauts joined a select group of Chicago-area business leaders at a fundraising gala highlighting the ISS and its unique role in engaging young explorers and the public alike.

DESTINATION STATION: RESEARCH TRIANGLE PARK

In early May, Destination Station (a NASA-led outreach effort to educate the public about the ISS) traveled to Research Triangle Park, North Carolina—an area with deep ties to the biomedical and agricultural sciences communities. Over the years, Destination Station has become a useful tool to educate businesses about the capabilities of the ISS and promote the notion that "space is closer than you think." During the trip, CASIS and NASA leaders (along with astronauts Rex Walheim and Doug Wheelock) met with representatives from the state of North Carolina, pharmaceutical companies Bayer and GlaxoSmithKline, the agricultural company Syngenta, and many others to discuss opportunities to leverage the ISS National Lab for innovative research.