National Aeronautics and Space Administration

NASA

A Researcher's Guide to:

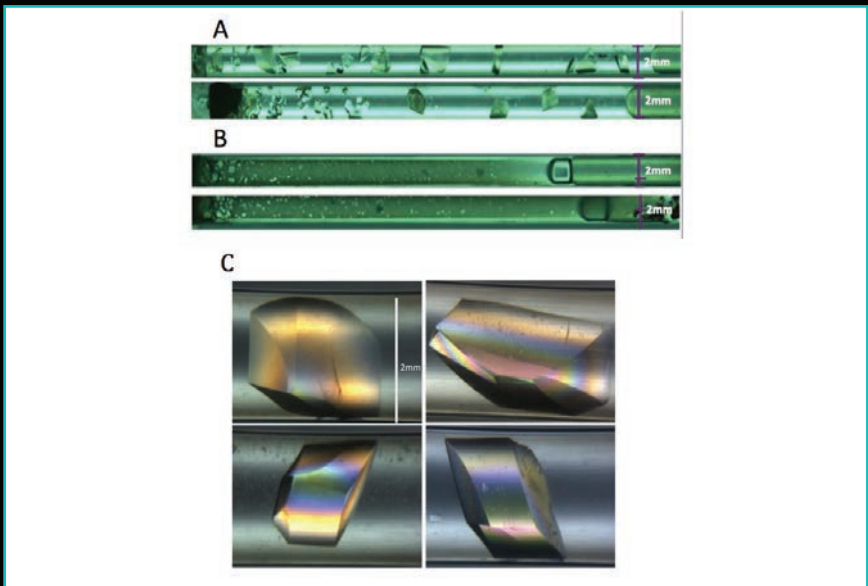INTERNATIONAL SPACE STATION

Macromolecular Crystal Growth
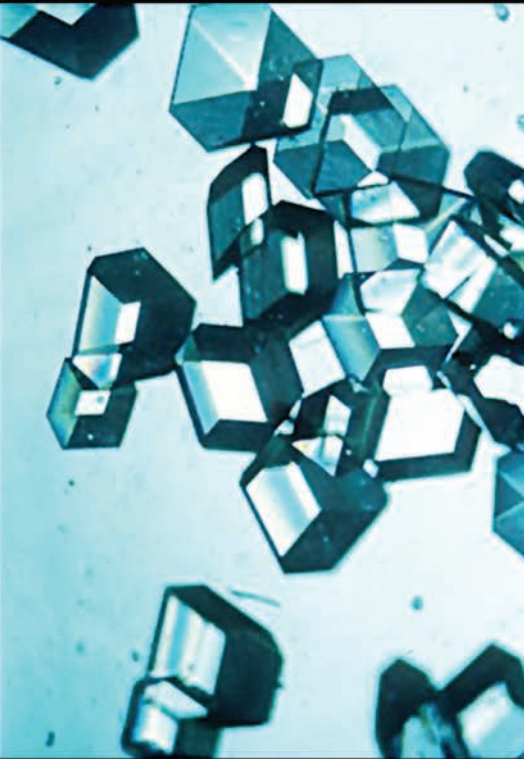
Cover and back cover:
a. Recombinant human insulin crystals grown during STS-95 within the Protein Crystallization Facility by the temperature induction batch method. (Photos courtesy of the University of Alabama at Birmingham; publication Smith, Ciszak et al. 1996.)
b. Enhanced Diffusion-controlled Crystallization Apparatus for Microgravity. (Photo courtesy of NASA's Marshall Space Flight Center.)
c. High Density Protein Crystal Growth. (Photo courtesy of the University of Alabama at Birmingham.)

The Lab is Open

Orbiting the Earth at almost 5 miles per second, a structure exists that is nearly the size of a football field and weighs almost a million pounds. The International Space Station (ISS) is a testament to international cooperation and significant achievements in engineering. Beyond all of this, the ISS is a truly unique research platform. The possibilities of what can be discovered by conducting research on the ISS are endless and have the potential to contribute to the greater good of life on Earth and inspire generations of researchers to come.

As we increase utilization of ISS as a National Laboratory, now is the time for investigators to propose new research and to make discoveries unveiling new knowledge about nature that could not be defined using traditional approaches on Earth.

CASIS-sponsored experiment flown to the International Space Station on SpaceX 3 and returned to Earth on SpaceX 4. Large protein crystals are needed for Neutron Diffraction structure determination. Quartz capillaries containing crystals of inorganic pyrophosphatase grown in space for about six months (A) and crystals grown on Earth (B). Capillaries are 2 mm in diameter. Typical crystals grown in space are shown under polarized light (C; Ng, Baird et al. 2015).

Unique Features of the ISS Research Environment

1. Microgravity, or weightlessness, alters many observable phenomena within the physical and life sciences. Systems and processes affected by microgravity include surface wetting and interfacial tension, multiphase flow and heat transfer, multiphase system dynamics, solidification, and fire phenomena and combustion. Microgravity induces a vast array of changes in organisms ranging from bacteria to humans, including global alterations in gene expression and 3-D aggregation of cells into tissue-like architecture.

2. Extreme conditions in the ISS environment include exposure to extreme heat and cold cycling, ultra-vacuum, atomic oxygen, and high-energy radiation. Testing and qualification of materials exposed to these extreme conditions have provided data to enable the manufacturing of long-life, reliable components used on Earth as well as in the world's most sophisticated satellite and spacecraft components.

3. Low-Earth orbit at 51 degrees inclination and at a 90-minute orbit affords ISS a unique vantage point with an altitude of approximately 240 miles (400 kilometers) and an orbital path over 90 percent of the Earth's population. This can provide improved spatial resolution and variable lighting conditions compared to the sun-synchronous orbits of typical Earth remote-sensing satellites.

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Why Macromolecular Crystal Growth and Why in Microgravity?

Although macromolecular crystals grown in microgravity consist of proteins, DNA, RNA and even whole viruses, the vast majority of macromolecular crystals have been proteins. There are over 100,000 proteins in the human body and an estimated 10 billion throughout the global environment. Proteins serve many functions, including the maintenance of structure, function and regulation of the body's tissues and organs, and provide for catalysis of chemical reactions, cell-to-cell signaling, and immune responses. To fully understand how they work and how they interact with each other, it is necessary to determine their 3-D structure. This is most often done through analysis of X-ray diffraction of quality crystals. A newer method, using analysis by neutron diffraction, determines the position of hydrogens within a protein structure and enables more accurate determination of the mechanisms of biochemical reactions taking place within and between proteins (Blakeley, Langan et al. 2008, Niimura and Bau 2008). Neutron diffraction requires very large quality crystals, greater than 1 millimeter^3 in volume, in most cases. Fewer than 100 unique neutron structures of proteins have been reported in the Protein Data Bank, as compared to over 90,000 X-ray diffraction structures. Figure 1 shows a neutron diffraction-derived structure of the protein Myoglobin. High-resolution data for X-ray diffraction and neutron diffraction structure determination requires crystals of high quality with few defects, and this is often the bottleneck for crystallographers. It is particularly difficult to grow high-quality crystals of membrane proteins that have the desired qualities, as evidenced by the fact that only 539 unique structures have been reported since the first structure was determined in 1985 (Deisenhofer, Epp et al. 1985). It is estimated that 20-30 percent of all genes in all genomes are integral membrane proteins (Kahsay, Gao et al. 2005) and that membrane proteins are the targets of over 50
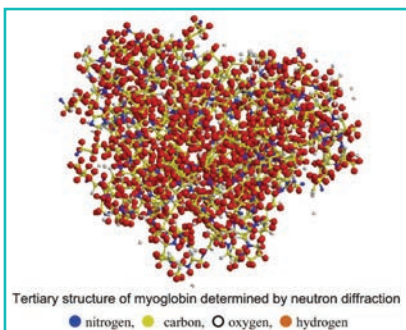

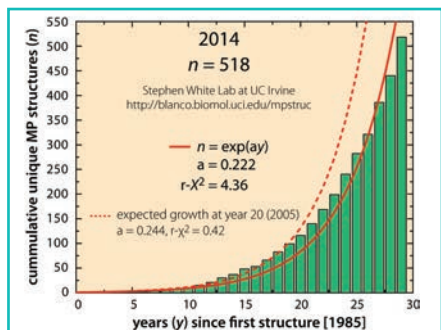Figure 1. "Neutron". (Licensed under Public Domain via Wikibooks. http://en.wikibooks.org/wiki/File:Neutron.jpg#/media/File:Neutron.jpg)

Figure 2. Cumulative Unique Membrane Protein Structures. (http://blanco.biomol.uci.edu/mpstruc/)

percent of all modern medicinal drugs (Overington, Al-Lazikani et al. 2006). Figure 2 illustrates the progress in membrane structure determination since 1985.

Based on the success of genomic sequencing, in 2000, two institutes of the National Institutes of Health (the National Institute of General Medical Sciences and the National Institute of Allergy and Infectious Diseases) collaborated in funding nine pilot research centers for high-throughput structural determinations. The goal of these projects was to determine novel structures having less than 30 percent identity in sequence to proteins whose structures had already been determined (Norvell and Berg 2007). This five-year effort was renewed and enlarged in 2005. Similar initiatives were begun in other countries as well. Although many protein structures have been submitted to the Protein Data Bank, the numbers were not as high as what was originally anticipated, and one of the bottlenecks, along with the production of soluble proteins, is successful crystallization (Grabowski, Chruszcz et al. 2009). A recent set of statistics for one of the most successful centers, the Northeast Structural Genomics Center, shows that 25,759 proteins have been cloned, and 6,407 proteins have been purified while only 1,480 of them have been crystallized successfully (23.3 percent; http://www.nesg.org/statistics.html). Likewise, the Midwest Center for Structural Genomics has 37,012 active targets and 3,175 crystals produced (8.5 percent). Of these, 1,843 structures have been determined (http://www.mcsg.anl.gov/).

Proteins and other macromolecules have been crystallized in microgravity experiments for over three decades. The first microgravity experiment in protein crystal growth was in 1981 when Littke conducted a six-minute microgravity experiment with β-galactosidase on the German TEXUS sounding rocket. Video from this experiment showed a laminar diffusion process rather than the turbulent convection that occurs on Earth (Littke and John 1984). Excellent discussions of the effects of growing macromolecular crystals in microgravity have been published and in press (Snell and Helliwell 2005, McPherson and DeLucas 2015).

Some characteristics of crystals that are recognized as measurements of quality include visual perfection and size, resolution limit, I/sigma ratio (in essence signal-to-noise ratio) and mosaicity. It is believed that factors affecting crystal growth in microgravity include lack of buoyancy-driven convection and lack of sedimentation. Pusey et al. illustrated the convection patterns (or growth plumes) of lysozyme crystals grown in Earth's gravity (Figure 3; Pusey, Witherow et al. 1988). On Earth, convective flows transport macromolecules to the surface of the growing crystal, while in microgravity, these buoyancy-driven convective flows are
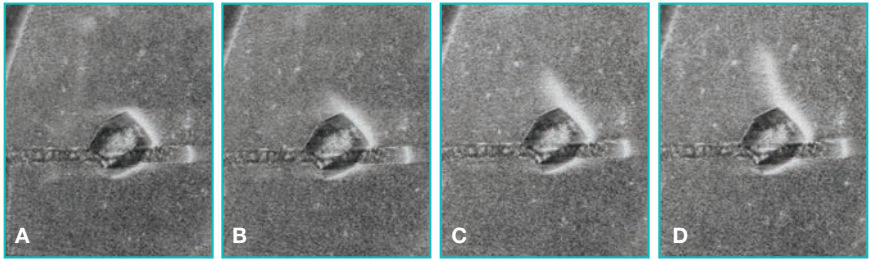
Figure 3. Schlieren photography shows sequential convective growth plume formation around a lysozyme crystal grown on Earth (Pusey, Witherow et al. 1988).

not present and the area around the crystal becomes depleted of macromolecules. Thus, addition of molecules to the growing crystal is governed only by diffusion. It has been hypothesized that the depleted area around a crystal causes slower growth, allowing the crystal to form with fewer imperfections and also impedes the addition of aggregates (because of the slower diffusion of larger molecules; Lin, Rosenberger et al. 1995; Lin, Petsev et al. 2001). This depletion zone was first visualized by McPherson and all using Mach-Zhender interferometry on a device called the Observable Protein Crystal Growth Apparatus (OPCGA; McPherson, J. Malkin et al. 1999), which was slated for use on the ISS but was canceled following the Space Shuttle *Columbia* disaster. More recently, these stable depletion zones around growing crystals have been visualized and recorded in experiments on the ISS in the Advanced Protein Growth Facility (APCF; Otalora, Garcia-Ruiz et al. 2002) and in the Nano Step experiment (Yoshizaki, Tsukamoto et al. 2013).
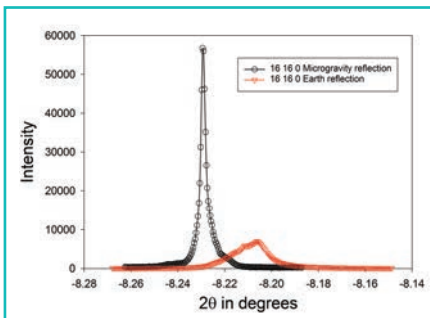
Figure 4. Comparison of Mosaicity of tetragonal Lysozyme crystals grown on the ground and in microgravity (Snell, Weisgerber et al. 1995).

Many published reports from microgravity macromolecular growth experiments have described crystals having much greater volume than any grown previously on the ground, which gave X-ray diffraction data of higher resolution and I/sigma over the entire resolution range. Table 1 provides a list of macromolecules (with references) for which crystal growth in microgravity provided significant improvement in the quality of data over crystals grown on Earth up to that time. The list is

not all-inclusive, since many experiments flown were for the benefit of commercial entities, and it is doubtful all of those results have been or will be published. Careful measurements of the mosaicity of crystals have also shown marked improvement for microgravity-grown crystals over those grown on Earth. Snell and his colleagues first reported this in 1995 with tetragonal lysozyme crystals grown on two separate shuttle missions, in which they demonstrated an improvement by a factor of three to four over Earth-grown crystals (Snell, Weisgerber et al. 1995; Figure 4). Similar results comparing microgravity crystals of aminoacyl-tRNA synthetase grown within dialysis reactors of the European Space Agency's (ESA's) Advanced Protein Crystallization Facility (APCF) on the shuttle STS-78 mission (Ng, Sauter et al. 2002) and with microgravity-grown Insulin crystals on the STS-95 mission (grown in the commercial Protein Crystallization Facility [PCF]; Borgstahl, Vahedi-Faridi et al. 2001).

Comparisons of crystals grown in microgravity with those grown on Earth under the same conditions and in the same equipment is not always the best comparison, since the best conditions for growth with gravity are often different than the best conditions in microgravity. Because of this, comparisons for published results were often between the best conditions seen in microgravity experiments compared with all of the conditions that had previously been used in Earth laboratories. That there are so many success stories is fairly remarkable because especially early in the space shuttle era, the hardware for microgravity experiments had relatively few slots to screen conditions for optimal crystal growth. So the comparisons were between a few conditions versus hundreds to thousands of conditions attempted on Earth.

The microgravity conditions aboard the space shuttle were not always optimal, because of crew activities, minor attitude adjustments and operation of equipment. Additionally, when accounting for the short time-frames of shuttle missions (usually 7-14 days) and the unforeseen delays in launches, it becomes even more remarkable that about 40% of the macromolecular crystals grown under microgravity were of better quality than those grown on Earth, based on the space-grown crystals' improved x-ray diffraction intensity, resolution and mosaicity (Judge, Snell et al. 2005). The successful samples represented 177 different macromolecules available for analysis within 63 missions. This group additionally reported that chances for success were much greater on missions dedicated to providing a microgravity environment than those that had crystallization experiments as secondary payloads to other activities, such as satellite launches and retrievals (55 percent success versus 34 percent), and that longer missions trended toward better results, but this was macromolecule specific. This bodes well for crystallization experiments on the ISS.

They also found that some macromolecules do better consistently in microgravity while some do not, an observation which has not yet been adequately explained. Another review of shuttle experimental results analyzed the number of flights of macromolecules versus an improvement in diffraction quality and reported about 20 percent of the macromolecules flown obtained the highest diffraction resolution to date. However, if only macromolecules that were flown more than once were considered, then the chance of producing better diffracting crystals increased to 35 percent. This illustrates that iterations of crystal growth in microgravity is highly important (Kundrot, Judge et al. 2001).

Brief History of Macromolecular Crystal Growth in Microgravity

Good reviews of macromolecular crystal growth in microgravity are available, so this will only be briefly discussed (Lorber 2002; Vergara, Lorber et al. 2003; Judge, Snell et al. 2005; Snell and Helliwell 2005; McPherson and DeLucas 2015). As noted above, macromolecular crystal growth in microgravity was first studied by Littke in 1981 aboard the TEXUS sounding rocket for six minutes in a liquid-liquid diffusion experiment, showing strictly laminar diffusion patterns (Littke and John 1984). Macromolecular crystal growth experiments were also included on some of the unmanned series of Russian Foton satellite missions including April 1988 (Trakhanov, Grebenko et al. 1991) and 1991, the Foton-3 KASHTAN experiment (Chayen 1995). In 2007, an ESA-sponsored mission on Foton-M3 provided the first flight of the Granada Crystallization Facility-2 (Gonzalez-Ramirez, Carrera et al. 2008). Other unmanned experiments included the Swedish Material Science Experiment Rocket (MASER) flown in 1989 with about seven minutes of microgravity (Sjölin, Wlodawer et al. 1991), and the China-23, carrying Crystallization of Organic Substances in Microgravity for Applied Research (COSIMA-1; Plass-Link 1990).

An experiment based on the TEXUS hardware was flown on STS-9 in 1983 and grew crystals of lysozyme and β-galactosidase (Littke and John 1986). The Vapor Diffusion Apparatus (VDA) first flew in 1985 (DeLucas, Suddath et al. 1986). The design of this hardware was meant to mimic the hanging drop vapor diffusion experiments most utilized for crystallization on Earth. Many drops were lost during this experiment, but subsequent refinements were made for later flights. The first flight with temperature control was STS-26, following the *Challenger* disaster, also utilizing the VDA hardware. From this point until about 2004, many shuttle flights had at least one macromolecular crystal growth experiment, and often two or more. Also, new designs and methods for crystallization in microgravity came quickly.
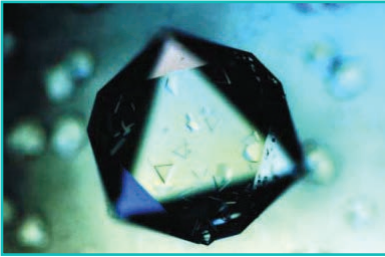
Figure 5. Satellite Tobacco Mosaic Virus crystal grown in Microgravity. (Photo courtesy of Dr. Alexander McPherson, University of California, Irvine)

Figure 6. "Protein Crystal Malic Enzyme." (Licensed under Public Domain via Wikimedia Commons. http://commons.wikimedia.org/wiki/File:Protein_Crystal_Malic_Enzyme.jpg#/media/File:Protein_Crystal_Malic_Enzyme.jpg.)

The Protein Crystallization Facility (PCF) utilizes a large-scale, temperature-based crystallization method containing 20-500 ml. It first flew on STS-37 in 1991 and has flown several times since then. Activation is by temperature ramping, and has been most used for growth of many crystals having uniform sizes (Long, DeLucas et al. 1994; Long, Bishop et al. 1996).

STS-42 (International Microgravity Laboratory), flown in 1992, was the first flight to be dedicated to the maintenance of a microgravity environment. On this flight, both VDA and the German Cryostat (liquid-liquid diffusion) hardware were flown. In the Cryostat, a Satellite Tobacco Mosaic Virus (STMV) crystal was grown that was 30 times the volume of any STMV crystal that had ever been grown on Earth and resulted in a structure of 1.8 angstroms (Figure 5; Larson, Day et al. 1998). On STS-50, flown in 1992, Dr. Larry DeLucas operated a glovebox experiment enabling iterative experiments to optimize conditions and practice such techniques as seeding and crystal mounting as well as real-time video transfer of data. On this flight, a malic enzyme crystal was grown, which improved diffraction from 3.2 angstroms to 2.6 angstroms (Figure 6; DeLucas, Long et al. 1994).

Newer designs for crystallization in microgravity began to appear beginning with STS-57 in 1993. Included in these new designs was ESA's APCF, which was temperature controlled, contained 48 individual growth chambers that could operate either in a batch, dialysis, liquid-liquid, or vapor diffusion mode and could also provide a video of the growth in 12 of the experiments, as well as a Mach-Zehnder interferometer available after 1996 (Snyder, Fuhrmann et al. 1991; Bosch, Lautenschlager et al. 1992; Vergara, Lorber et al. 2003). The capacities of the APCF were later expanded in 1999 (STS-95) to include the Long Protein-Chamber

Free Interface Diffusion reactor utilizing a counter diffusion technique. In total, the APCF was flown on six missions including once on the ISS. An excellent example of the possibilities of microgravity for the growth of membrane proteins is shown in Figure 7. This is the membrane protein complex Photosystem I, crystallized in the APCF dialysis mode, which produced a crystal that was 4 mm in length, and 1.5 mm in diameter, and formed the basis for an improved crystal structure (Klukas, Schubert et al. 1999; Fromme and Grotjohann 2009).

The space shuttle also docked with the Russian Space Station Mir and carried macromolecular crystal growth experiments. In 1989, a vapor diffusion apparatus was used to crystallize chicken egg white lysozyme and D-amino transferase, producing crystals which were larger and diffracted somewhat better than those grown in Earth hardware (Stoddard, Strong et al. 1991). This device used a sitting drop rather than the hanging drop method used in the VDA. A more evolved sitting drop hardware was developed called the Protein Crystallization Apparatus for Microgravity (PCAM; Carter, Wright et al. 1999), which first flew in 1994 as a handheld device and grew into a hardware that accommodated many guest investigators and flew 13 times. Although crystals of many different proteins were grown in the PCAM, one striking example is shown in Figure 8 of a manganese superoxide dismutase crystal that was 80 times larger than any that had grown before
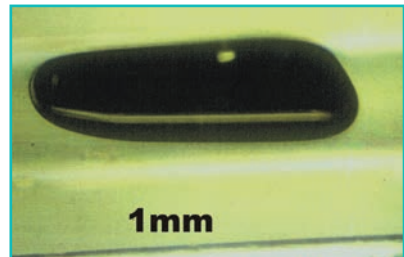
Figure 7. Large, single crystal of Photosystem I, grown during USML-2 in APCF by dialysis method (Fromme and Grotjohann 2009).
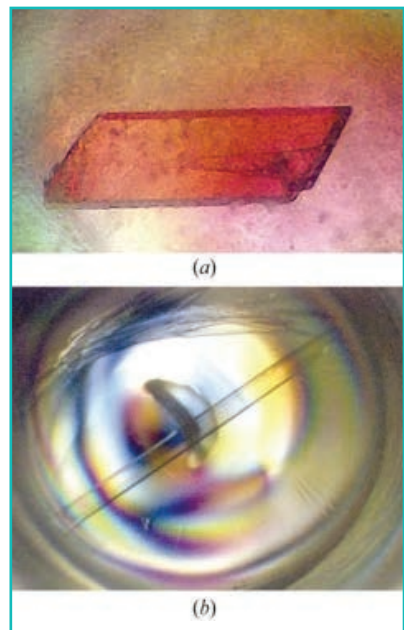
Figure 8. Examples of microgravity-grown MnSOD crystals in the PCAM crystallization chamber. (a) Crystal with dimensions 0.45 x 0.45 x 1.45 mm. The pink color is due to oxidized manganese in the active site (not ever seen in the thin crystals grown on Earth). (b) An example of crystals limited in size to 3 mm in length by the drop volume (Vahedi-Faridi, Porta et al. 2003).

Figure 9. X-ray diffraction analysis. Credit: Dr. Alex McPherson, University of California, Irvine.

on Earth (Vahedi-Faridi, Porta et al. 2003). The PCAM could hold 378 samples in a temperature controlled locker or 504 samples without temperature control.

The Gaseous Nitrogen Dewar (GN2) was first designed for a flight on Mir, since it required no temperature control and no crew time (Koszelak, Leja et al. 1996). This consisted of many sealed Tygon tubes with separately frozen precipitant and protein solutions. These were contained in a liquid nitrogen dewar and as they gradually thawed, liquid-liquid diffusion occurred and the proteins crystallized. This first experiment contained 183 samples of 19 proteins, but later refinements included many more samples,

thereby enabling optimization of growth conditions, and many samples were devoted to student education projects. This hardware flew many times as the Enhanced Gaseous Nitrogen (EGN) Dewar. Figure 9 shows pictures of some of the protein crystals that were grown on the Mir GN2 experiment. The second hardware designed for Mir is the Diffusion-controlled Crystallization Apparatus for Microgravity (DCAM), which also required no activation or deactivation by the crew. The DCAM sample chamber consisted of two cells holding precipitant and protein, which are separated by a gel plug through which they slowly equilibrate. On the first flight of DCAM, which occurred on STS-73 as a proof of concept, a crystal of nucleosome core particle grew that yielded the highest resolution to date (Figure 10 – Carter, Wright et al. 1999; Harp, Hanson et al. 2000). The DCAM hardware flew seven times, and a second-generation hardware (EDCAM) was designed and built, but not flown up to this time. A new and larger vapor diffusion apparatus,

the High Density Protein Crystal Growth (HDPCG) was designed by the University of Alabama at Birmingham, to take the place of the VDA and VDA-2 (which had a triple-barrel syringe). This hardware fits into a Microgravity Experiment Research Locker Incubator (MERLIN) and can hold up to 1,008 vapor diffusion samples. It flew two times to the ISS for NASA through 2002 and then again in 2014.

Around 2004, NASA-sponsored missions in macromolecular crystal growth were suspended until quite recently, but ESA, JAXA and Russia continued the research effort and kept developing new hardware and diagnostics. The ESA Granada Crystallization Facility (GCF) utilizes a counter-diffusion technique for crystallization in capillary tubes (Otalora, Gavira et al. 2009). These tubes are contained in a Granada Crystallization Box (GCB), which holds a maximum of six capillaries, with the GCF holding 23 GCBs (138 samples total). This flew on two sortie missions to the ISS, then JAXA (now NASDA), used it for nine missions between 2003 and 2009. The NASDA experiments with GCF were performed in collaboration

Figure 10. A. Nucleosome Core Particle crystal, grown in DCAM on STS-73. B. The structure of the protein was determined using the crystals grown in space to a 2.5 angstrom resolution (Harp, Hanson et al. 2000), PDB. http://www.rcsb.org/pdb/explore.do?structureId=1eqz.

with the Russian space agency Roscosmos in the Zvezda service module. NASDA then developed their own hardware called Protein Crystallization Research Facility (PCRF), which is located within the Japanese module Kibo (but still launched by Roscosmos). This new generation of counter-diffusion hardware is said to hold about 12 times the number of proteins. NASDA has had many success stories using the GCF and then the PCRF, but one particularly exciting example is the
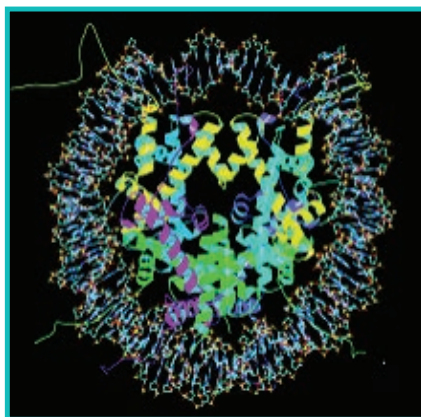
crystallization and structure determination of an inhibitor complexed with the protein prostaglandin D synthase. This protein is important in allergies and other inflammations, but is also believed to cause muscle necrosis in Duchenne Muscular Dystrophy. Results from microgravity experiments produced the highest resolution structure for this protein. This structure was then used as a template to design a more potent inhibitor and perhaps a treatment (Aritake, Kado et al. 2006; Mohri, Aritake et al. 2009; Tanaka, Tsurumura et al. 2011).

The APCF, described above, last flew in 2001 and was replaced with the Protein Crystallization Diagnostic Facility (PCDF), which is located in the ESA Columbus Laboratory since 2008. This facility has been used for understanding the phenomena associated with crystallization processes (Pletser, Bosch et al. 2009; Patiño-Lopez, Decanniere et al. 2012). The ESA Protein Microscope for the International Space Station (PromISS) facility was developed for ISS as well. One operation took place within the U.S. Microgravity Science Glovebox during a sortie mission on Expedition 12. Complete data sets of 17 crystals of ferritin grown in PromISS by a counter-diffusion method were compared with complete data sets of 18 crystals grown under the same conditions on Earth. Statistical analysis was performed of 63 parameters commonly used as indicators of X-ray data quality, and it was clearly indicated that the space crystals were of superior quality (Maes, Evrard et al. 2008).

NASA and the Center for the Advancement of Science in Space (CASIS) have resumed macromolecular crystal growth experiments within the last few years. CASIS sponsored a microfluidic experiment using a commercial Plug MakerTM/ CystalCardTM system (Protein BioSolutions), and it was carried to the ISS aboard the SpaceX Dragon capsule in 2013. This experiment included 25 Crystal Cards containing about 10,000 individual experiments within two NanoLabs (NanoRacks). During preparation of the experiment, protein, buffer and precipitant are mixed in nanoliter quantities in gradient concentrations. Each droplet of mixture is separated from the next by a biologically inert fluorocarbon, thus giving 10-20 nanoliter microbatch-style crystallization plugs within a small channel. Sixteen out of 25 cards from microgravity contained crystals while only 12 out of 25 of those on Earth had crystals (Gerdts, Elliott et al. 2008, Carruthers; Gerdts et al. 2013). This hardware has the advantage of high numbers of screening conditions of a multitude of proteins using very small volumes. The GCF, described above, was used again by multiple investigators on the ISS in 2014, sponsored by CASIS. One of the investigations, led by Joseph Ng, University of Alabama in Huntsville,

produced unusually large crystals of inorganic pyrophosphatase for neutron diffraction studies (Ng, Baird et al. 2015).

A NASA-sponsored experiment using a modified version of the HDPCG (described above) was carried out on the ISS in 2014. The experiment consisted of 360 vapor diffusion cells each at 4°C and 20°C, 840 liquid-liquid diffusion capillaries at 4°C and 900 liquid-liquid diffusion capillaries at 20°C. The total number of proteins flown was 96, at various conditions. Additionally, many of the capillaries contained experiments from a Science, Technology, Engineering, and Math (also known as STEM) competition between students from 10 different high schools. Analysis of results is ongoing.

Student Involvement

As noted above, the most recent high-volume crystallization experiment on ISS (HDPCG) involved students from 10 different high schools. Over the years that the EGN Dewar flew, over 50,000 students and 1,090 teachers from 320 schools across 36 states and Puerto Rico had direct involvement with macromolecular crystal growth through learning curriculums. Moreover, 420 of these students plus 260 of their teachers from 125 schools in 10 states participated in the flight program including flight sample-loading workshops and launch activities. These efforts as well as other high school programs, such as those sponsored by the Keck Center for Molecular Structure at California State University and the Lind laboratory at the University of Toledo, plus workshops sponsored by the American Crystallographic Association are all designed to promote the enthusiasm of students for science and technology, and perhaps to inspire the next generation of crystallographers (Kantardjieff, Lind et al. 2010).

What Should Principal Investigators Know About Conducting Research on ISS?

Supporting research in science and technology is an important part of NASA's overall mission. NASA solicits research through the release of NASA Research Announcements (NRA), which cover a wide range of scientific disciplines. All NRA solicitations are facilitated through the Web-based NASA Solicitation and Proposal Integrated Review and Evaluation System (NSPIRES; http://nspires.nasaprs.com/external/). Registering with NSPIRES allows investigators to stay informed of newly released NRAs and enables submission of proposals. NSPIRES supports the entire lifecycle of NASA research solicitations and awards, from the release of new research calls through the peer review and selection process.

In planning the scope of their proposal, investigators should be aware of available resources and the general direction guiding NASA research selection. NASA places high priority on recommendations from the 2011 National Research Council's NRC Decadal Survey, which placed emphasis on hypothesis-driven spaceflight research. In addition, principal investigators (PIs) should be aware that spaceflight experiments may be limited by a combination of power, crew time or volume constraints. Launch and/or landing scrubs are not uncommon, and alternative implementation scenarios should be considered in order to reduce the risk from these scrubs. Preliminary investigations using ground-based simulators may be necessary to optimize procedures before spaceflight. Also, many experiments require unique hardware to meet the needs of the spaceflight experiment. To understand previous spaceflight studies, prospective PIs should familiarize themselves with the NASA ISS Program Science Office database, which discusses research previously conducted on the ISS, including that of the International Partners. A detailed catalog of previous, current and proposed experiments, facilities, and results, including investigator information, research summaries, operations, hardware information, and related publications is available at www.nasa.gov/iss-science through the NASA ISS Program Office. Additionally, details pertaining to research previously supported by the Space Life and Physical Sciences Research and Applications Division of NASA's Human Exploration and Operations Mission Directorate can be located in the Space Life & Physical Sciences Research and Applications Division Task Book in a searchable online database format at: https://taskbook.nasaprs.com/Publication/welcome.cfm.

Macromolecular Crystal Growth Experiments – Lessons Learned

When planning macromolecular crystal growth experiments bound for the ISS, there are some lessons learned from previous missions.

Macromolecule purity, homogeneity and monodispersity

Since flight experiments require so much in the way of time, energy, paper work, and expense, it is a necessity to spend the extra effort on making sure one's favorite macromolecule(s) are as pure, homogeneous, and monodisperse as possible. Monodispersity can be measured using light, X-ray or neutron scattering procedures.

Hardware choices

As noted above, the best conditions for growing crystals on the ground are not necessarily the best conditions in a microgravity environment. After determining the best conditions obtained on Earth, this should be used as a starting point for bracketing conditions in microgravity. In general, nucleation and crystal growth is slower in microgravity than it is on Earth.

It is necessary to know the limitations of the macromolecule and its crystals as well. Some of the hardware described in this document requires freezing of proteins and then thawing prior to crystallization. This method can be quite good if the macromolecule is not degraded by freezing, since launches are sometimes delayed and frozen samples will not need to be re-loaded prior to launch. Additionally, for very long flights, samples can be thawed at an appropriate time point for optimal crystal growth, since some crystals will degrade over time. If possible, it would be good practice to try more than one method of crystallization, such as vapor diffusion and liquid-liquid diffusion.

Another consideration is temperature control. If the macromolecule is stable over a wide range of temperatures, then this will not be of consequence. If, however, it is temperature sensitive or a temperature gradient is the method of choice for crystallization, then it will be necessary to use hardware that is carefully temperature controlled. Although not reported frequently, it is also possible in hardware where there is a liquid-air interface, such as vapor diffusion, that the crystal may be subjected to damage that is due to vibration effects or re-entry.

Sample volumes required

Enough sample volume for re-load if a launch gets delayed is highly desirable (unless frozen samples are used). One also needs to have enough for control experiments on Earth using equivalent hardware and sample conditions. Also, using the same macromolecule batch for all needs (flight samples, reloads and controls) is much better if this can be accomplished, even if small batches have to be pooled to obtain one large batch. It may be desirable to use hardware that can accommodate a smaller sample if the macromolecule is very difficult to purify in large quantities, and therefore very expensive. However, it is possible that a very small sample in a vapor diffusion apparatus could evaporate during the mission, and if one desires a very large crystal for neutron diffraction, the size of the crystal can only get as large as there are macromolecules available to fill it.

Plan control experiments

In many cases, it is best to start control experiments a few days to a week after activation of the flight experiment, so that it is possible to follow closely the conditions to which the flight samples are subjected. There are a number of steps in the flight schedule over which the investigator has no control:

- From loading to launch.

- Transfer of experiment to ISS.

- Activation of experiment.

- Deactivation of experiment.

- Return flight to Earth.

Crystallization conditions

All chemicals that are flown on the ISS must be analyzed for toxicity by the Toxicology group at NASA's Johnson Space Center. This normally takes a while for the group to go through the entire list, so it is best to determine the best crystallizing conditions as soon as possible. One must bear in mind that chemicals that are too toxic, even in small quantities, may need to have additional levels of containment and thus may affect which hardware can be utilized.

Multipurpose Facilities
Available on the ISS

European Drawer Rack (EDR):

EDR supports seven Experiment Modules (EMs), each with independent cooling power and data communications as well as vacuum, venting and nitrogen supply, if required.
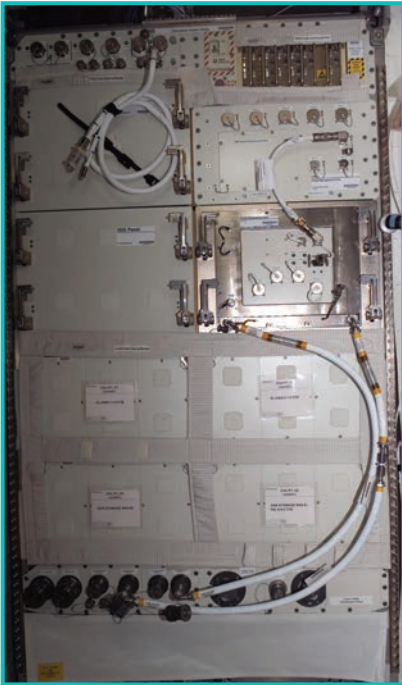
The European Drawer Rack, installed in the Columbus laboratory. Image was taken during Expedition 16.

EXpedite the PRocessing of Experiments for Space Station (EXPRESS) Racks:

EXPRESS Racks is a multipurpose payload rack system that provides structural interfaces, power, data cooling, water, and other items needed to operate experiments in space.

Crew member Naoko Yamazaki works to transfer EXpedite the PRocessing of Experiments to Space Station Rack 7 from the Multi-Purpose Logistics Module during STS-131/Expedition 23 Joint Docked Ops.

General Laboratory Active Cryogenic ISS Experiment Refrigerator (GLACIER):

GLACIER provides a double middeck-locker-size freezer/refrigerator for a variety of experiments that require temperatures ranging between +4°C (39°F) and -160°C (-301°F). The GLACIER is compatible with the EXPRESS rack. It is part of the cold-stowage fleet of hardware that includes the Minus Eighty Degree Laboratory Freezer for ISS and the MERLIN. The GLACIER incorporates a cold volume sample storage area of 23.1 cm (10.75 in.) x 27.94 cm (11.00 in.) x 41.91 cm (16.5 in.). It is capable of supporting 10 kg (22 lb) of experiment samples and has an internal cold volume of 20 L. The GLACIER can maintain a temperature of -160°C (-256°F) for 6 to 8 hours without power if it has been operating at -160°C (-301°F) prior to the power outage.

View of the general Laboratory Active Cryogenic ISS Experiment Refrigerator within EXpedite the PRocessing of Experiments to Space Station Rack 6 in the U.S. Destiny Laboratory during Expedition 18.

Gaseous Nitrogen Freezer (GN2):

NASA's Kennedy Space Center Gaseous Nitrogen Freezer (GN2) is a passive freezer container (requires no power). It is designed to hold samples at cryogenic temperatures (-196 °C) for between 21 and 35 days, depending on the flight configuration and use scenario. The GN2 can be used to transport frozen samples to and from orbit and is certified to fly on the International Space Station.

The sample area inside the internal tank can hold up to four cylinders 6.0 in. long and 3.7 in. in outer diameter. In addition, one of the freezer compartments will be filled preflight with an insert of the same absorbent material to increase the thermal mass of the system. The additional insert is wrapped with cotton cloth to contain any particulate.

NASA's Kennedy Space Center (KSC) Gaseous Nitrogen Freezer with lid removed. (Image is courtesy of KSC.)

Single-locker Thermal Enclosure System (STES):

The STES is a single-locker equivalent, thermally controlled experiment module capable of operating as either a refrigerator with a minimum set point temperature as low as 4.0°C, or as an incubator with a maximum set point temperature of 40.0°C. The STES can maintain a constant set point temperature, or it may be pre-programmed to step through a series of temperatures. The STES can be operated remotely when installed in an EXPRESS rack or manually by the crew through push buttons and a LCD located on the front panel.

Close-up of the Single-locker Thermal Enclosure System in Express Rack 4 aboard ISS, during Expedition 7.

Commercial Refrigerator Incubator Module – Modified:

The Commercial Refrigerator Incubator Module - Modified (CRIM-M) is a single middeck locker equivalent thermal incubator used for investigations requiring thermal control between 4 and 40°C. The CRIM-M provides thermal performance by power only and is designed to communicate with the EXPRESS racks for remote operations. The internal compartment provides a 28-V power receptacle and can control temperature to within 0.5°C. The internal payload volume dimensions are 17.3 cm x 25.9 cm x 41.9 cm with a maximum allowable experiment weight of 11.25 kg.

The Commercial Refrigerator Incubator Module – Modified (CRIM-M) is a single, middeck-locker equivalent thermal incubator for payloads requiring thermal control between 4 and 40°C. Image is courtesy of CBSE Engineering Division.

Microgravity Experiment Research Locker Incubator (MERLIN):

Microgravity Experiment Research Locker Incubator (MERLIN) provides a single, middeck, locker-sized EXPRESS Rack compatible freezer/refrigerator or incubator that can be used for a variety of experiments. Temperature range for MERLIN is -20°C (-4°F) to + 48.5°C (+119°F).

View of Microgravity Experiment Research Locker/ INcubator on the forward middeck on Space Shuttle Endeavour. *Photo was taken during STS-123 / Expedition 16 joint operations.*

Polar:

Polar is a cold stowage managed facility that provides transport and storage of science samples at cryogenic temperatures (-80°C) to and from ISS. Polar operates on 75 W supplied power and uses air cooling as its heat-rejection method. Polar can accommodate up to 12.75 liters of sample volume and 20 lbm including sample support equipment.
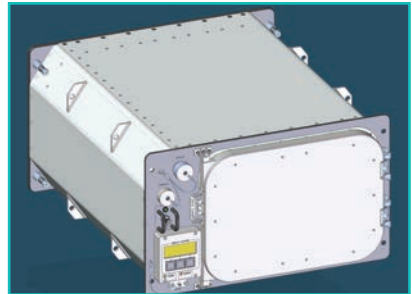
Polar Flight Assembly CBSE-F10120-1. Image provided by the University of Alabama at Birmingham Center for Biophysical Sciences and Engineering.

Kubik:

Kubik consists of a small controlled-temperature volume, which functions both as an incubator and cooler (6°C to 38°C temperature range). Self-contained automatic experiments, including crystallization experiments, seeds, cells, and small animals, are performed using power provided by the facility. A centrifuge insert permits simultaneous 1-g control samples to run with microgravity samples. There are no data or command communication possibilities between the experiments and Kubik.

Cosmonaut Salizhan S. Sharipov pictured with the Kubik incubator aboard the International Space Station. (Image is courtesy of ESA.)

Commercial Generic Bioprocessing Apparatus (CGBA):

The CGBA provides programmable, accurate temperature control for applications ranging from cold stowage to customizable incubation. It provides automated processing for biological experiments. The CBGA is designed to be installed in the EXPRESS rack for in-orbit operation.
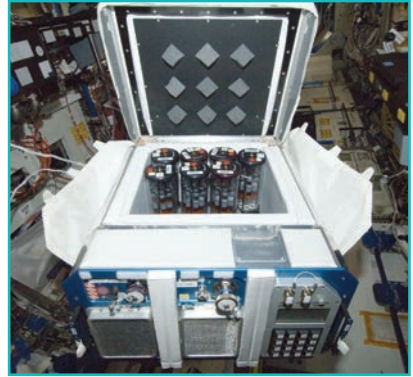
Photograph of Commercial Generic Bioprocessing Apparatus during Increment 33 showing open containment volume and sample canisters. (Image courtesy of NASA.)

Microgravity Science Glovebox (MSG):

The Microgravity Science Glovebox (MSG) facility on International Space Station has a large front window and built-in gloves, creating a sealed environment to contain liquids and particles in microgravity for science and technology experiments. More than 30 investigations have used the versatile Glovebox, everything from material science to life sciences. Ports are equipped with rugged, sealed gloves that can be removed when contaminants are not present, and video and data downlinks allow experiments to be controlled from the ground. Researchers also use MSG to test small parts of larger investigations and try out new equipment in microgravity.

Expedition 8 Commander and Science Officer Michael Foale conducts an inspection of the Microgravity Science Glovebox.

Light Microscopy Module (LMM):

Light Microscopy Module (LMM) is housed within and used in conjunction with the glovebox in the Fluids Integrated Rack. Images provided by the LMM can provide data to scientists and engineers to help understand the forces that control the organization and dynamics of matter at microscopic scales. The LMM microscope is capable of using most standard Leica objectives. The present in-orbit compliment includes: 2.5x,10x, 20x, 40x, 50x, 63x, 63x oil, and 100x oil objectives. New or different objectives may also be flown as needed. The LMM contains a digital black-and-white, low-noise scientific camera. 3-D confocal (point illumination) upgrades are scheduled for 2015-2016.

Light Microscopy Module. Image courtesy of NASA's Glenn Research Center.

Nanoracks Microscopes:

The NanoRacks Microscopes facility includes three commercial off-the-shelf optical and reflective microscopes. They utilize plug and play USB technology and allow crew members to analyze and digitally transfer images of ISS in-orbit samples.

NanoRacks Microscope-3 is an off-the-shelf USB microscope. Image is courtesy of NanoRacks LLC.

Hardware Designed for Crystallization of Macromolecules

Kristallizator (Crystallizer):

Kristallizator (Crystallizer) allows the growth of large protein crystals in orbit and allows better determination of the 3-D structure of the crystals, with applications in applied biology, medicine, and pharmacology. It is operated in the CRYOGEM-03 cooler.

Kristallizator (Crystallizer). (Image courtesy of the Russian Federal Space Agency.)

Image Processing Unit (IPU):

The Image Processing Unit (IPU) is a JAXA subrack facility that receives, records and downlinks experiment image data for experiment processing. The IPU is housed in the Ryutai (fluid) experiment rack with the Fluid Physics Experiment Facility, Solution Crystallization Observation Facility (SCOF), and PCRF.

Image Processing Unit. (Image is courtesy of JAXA.)

Solution Crystallization Observation Facility (SCOF):

SCOF is a JAXA subrack facility, located in the Ryutai (fluid) Rack, which will investigate morphology and growth of crystals. The SCOF is equipped with several microscopes to simultaneously measure changes in morphology and growth conditions (temperature and concentration) of crystals. The SCOF has an amplitude modulation microscope and is equipped with two wavelength interference microscopes to simultaneously measure changes in morphology and growth conditions.

Solution Crystallization Observation Facility. Image courtesy of JAXA.

Protein Crystallization Research Facility (PCRF):

The PCRF is a JAXA subrack facility, located in the Ryutai (fluid) Rack, which will investigate protein crystal growth in microgravity. The PCRF can accommodate six cell cartridges. Each cell cartridge can accommodate a motor drive and Peltier elements, from which activation and termination timing, as well as temperature profiles, can be freely designed by the investigator. An experimental profile appropriate for each protein can be established. A CCD camera enables real-time monitoring of crystal growth. PCRF Peltier elements installed to cartridges provide temperature profiles suitable for target proteins from 0 to 35°C. PCRF can accommodate six cell cartridges containing 10 to 16 wells per cartridge that can hold 10 to 500 microliters per well. The following methods will be used to create crystals: Vapor Diffusion; Batch; Membrane and Liquid-liquid Diffusion.

Astronaut Tim Kopra, Expedition 20 flight engineer, works at the Protein Crystallization Research Facility in the Kibo laboratory of the International Space Station.

NanoRacks-Protein Crystal Growth-1:

NanoRacks-Protein Crystal Growth-1 (NanoRacks-PCG-1) is a proprietary protein crystal growth experiment that utilizes state-of-the-art, on-the-ground PCG procedures and hardware. NanoRacks-PCG-1 uses different PCG solutions in small crystal slides to grow protein crystals in microgravity. The slides are launched frozen, thawed in orbit to allow crystal growth, examined while in orbit and then returned.

NanoRacks-Protein Crystal Growth-1 (NanoRacks-PCG-1) is a proprietary protein crystal growth housed inside NanoRacks Module-19. Image is courtesy of Carl W. Carruthers, Jr.

Granada Crystallization Facility (GCF):

The GCF is a multiuser facility designed to conduct crystallization experiments of biological macromolecules in microgravity using a counter diffusion technique inside capillaries. The capillaries are enclosed within Granada Crystallization Boxes (GCBs). It does not require crew time during operations and is a passive device (no electrical power necessary). GCF can hold 300 crystallization experiments. It works under diffusion-controlled mass transport, and the technique automatically "searches" for the optimal crystallization conditions.
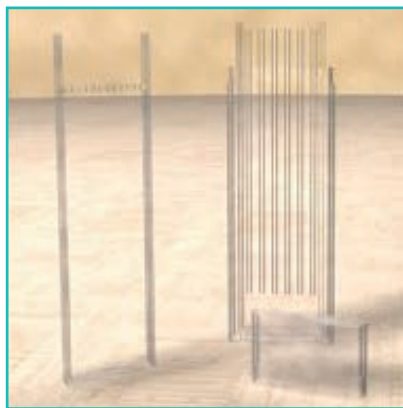
View of the Granada Crystallisation Facility. (Photo courtesy of ESA.)

## Protein Crystallization Diagnostics Facility (PCDF):

The Protein Crystallization Diagnostics Facility (PCDF) is a multi-user facility for the investigation of protein crystal growth and other biological macromolecules under microgravity. Crystallization experiments using the dialysis or the batch method can be performed. PCDF is designed for accommodation in the EDR on the ISS. The facility possesses diagnostic tools (microscope, optics, interferometers, video camera) that provide in-depth knowledge and understanding of protein crystal growth processes under microgravity.

The Protein Crystallization Diagnostics Facility.

High Density Protein Crystal Growth (HDPCG):

HDPCG holds 1,008 vapor diffusion samples or a mix of vapor diffusion and liquid-liquid diffusion samples. HDPCG is housed in MERLIN for active thermal control (+4°C to +48°C). It is suitable for crystallization of a large variety of aqueous and membrane proteins involved in critical biological processes and others that play key roles in infectious and chronic diseases. One advantage of this hardware design is the ability to delay activation of the vapor diffusion process until the hardware reaches orbit. This ensures that the crystallization process is not initiated on the Launchpad.
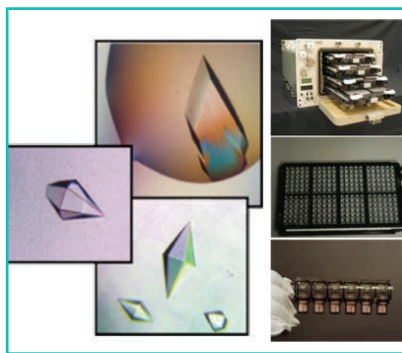
High Density Protein Crystal Growth. (Image courtesy of the University of Alabama at Birmingham.)

Enhanced Diffusion-Controlled Crystallization Apparatus for Microgravity (EDCAM):

Production of aqueous and membrane protein crystals with improved size and perfection using liquid/liquid diffusion and dialysis growth methods in support of structure determination by x-ray and neutron crystallography. Counter-diffusion cells can be individually programmed to control rate of approach to super-saturation over periods from several days to months. EDCAM can be flown in thermal carrier or in passive stowage depending on target investigations. EDCAM is self-activating with no crew interaction and contains eight Counter-Diffusion Cells per cylinder and 11 cylinders per thermal carrier. Counter-Diffusion Cells could be used for a variety of experiments including chemical, colloidal, gelation, cell culture additives and/or fixation studies. The cylinder can be transferred to the Glovebox and the internal experiments removed for manipulation, activation or viewing.

The Enhanced Diffusion-Controlled Crystallization Apparatus for Microgravity Image Courtesy of NASA's Marshall Space Flight Center.

Process for Payload Development

Following selection of an experiment for spaceflight, the PI will work with a payload integrator or hardware developer to define the most suitable hardware, and determine if hardware needs to be created or modified. The research team in combination with payload integrations will establish the specific laboratory requirements needed to support the experiment. Through these collaborative efforts, concerns such as crew procedures and crew training, the need for spare parts and/or contingencies involving hardware, and stowage requirements of the samples will be addressed and resolved. It is highly recommended that the PI perform a series of investigations using the identical hardware and under configuration and control conditions similar to those anticipated in flight prior to the launch. This will prevent unforeseen issues with the hardware and allow specific mission constraints to be defined and mitigated prior to the experiment implementation once aboard the ISS. It is also within this time frame that the science team needs to characterize the details involved with their synchronous ground controls. The PI's team should also have finalized all post-landing procedures, including crystal storage and transport, and data acquisition prior to the launch.

Another option to flying one's experiments is through the CASIS (http://www.iss-casis.org). CASIS is a nonprofit organization tasked by the U.S. Congress and NASA with promoting and enabling research on ISS. CASIS can be used for all stages of payload development and can match PIs with implementation partners (Table 2) who can provide heritage hardware or new flight packages.

Contacts for Macromolecular Crystal Growth Experiments:

CASIS:

Michael S. Roberts, Ph.D.   mroberts@iss-casis.org
                                   michael.s.roberts@nasa.gov

Jonathan Volk, Ph.D.   jvolk@iss-casis.org

NASA/Space Life & Physical Sciences Division:

Francis P. Chiaramonte, Ph.D.   francis.p.chiaramonte@nasa.gov

Macromolecule	PDB Identifier	Apparatus	Method	Data
Purine nucleoside phosphorylase	IULA	VDA	VD	(Ealick, Babu et al. 1991)
Interferon γ	1HIG	VDA	VD	(Ealick, Cook et al. 1991)
Human Serum Albumin	1UOR	VDA	VD	(He and Carter 1992)
Phospho carrier protein FAB complex	1JEL	VDA	VD	(Prasad, Sharma et al. 1993)
Factor D	1DSU	VDA	VD	(Narayana, Carson et al. 1994)
Fkbp12 (immunosuppressant binding protein)	1FKK	VDA	VD	(Wilson, Yamashita et al. 1995)
Rec. Human Insulin with phenolic inhibitor	1BEN	PCF	Temp	(Smith, Ciszak et al. 1996)
Antithrombin III	2ANT	PCAM	VD	(Skinner, Abrahams et al. 1997)
Satellite tobacco mosaic virus	1A34	CRYOSTAT	FID	(Larson, Day et al. 1998)
Bacteriophage Lambda Lysozyme	1AM7	PCAM	VD	(Evrard, Fastrez et al. 1998)
Eco R1 Endonuclease	1CKQ/ 1CL8	PCAM	VD	(Carter, Wright et al. 1999)
EF-Hand parvalbumin	2PVB	PCAM	VD	(Declercq, Evrard et al. 1999)
Hen Egg White Lysozyme	1BWJ	APCF	DIA	(Dong, Boggon et al. 1999)
Catalase	4BLC	EGN	FID	(Ko, Day et al. 1999)
Photosystem I	1C51	APCF	DIA	(Klukas, Schubert et al. 1999)
Collagenase	2HLC	APCF	VD	(Broutin-L'Hermite, Ries-Kautt et al. 2000)
Nucleosome Core Particle	1EQZ	DCAM	DIA	(Harp, Hanson et al. 2000)
Canavalin	1DGW	APCF	FID	(Ko, Day et al. 2000)
Monoclinic Egg White Lysozyme(neutron diffraction)	n/a	DCAM	DIA	(Ho, Declercq et al. 2001)
Lysozyme	1IEE	APCF	FID	(Sauter, Otalora et al. 2001)
Proteinase K (serine protease)	1IC6	VDA	VD	(Betzel, Gourinath et al. 2001)
Human Bence-Jones protein	1LGV	LMA	VD	(Alvarado, DeWitt et al. 2001)
[(Pro-Pro-Gly)10]3 Collagen-like polypeptide	1K6F	APCF	DIA	(Berisio, Vitagliano et al. 2002)
Mistletoe lectin	1M2T	HDPCG	VD	(Krauspenhaar, Rypniewski et al. 2002)
Alcohol dehydrogenase	1JVB	APCF	DIA	(Esposito, Sica et al. 2002)
NAD synthetase	1KQP	VDA	VD	(Symersky, Devedjiev et al. 2002)
Aspartyl-tRNA synthetase	1L0W	APCF	DIA	(Ng, Sauter et al. 2002)
Apocrustacyanin C1	1OBQ	APCF	VD	(Habash, Boggon et al. 2003)
Myoglobin	1NAZ	HDPCG	VD	(Miele, Federici et al. 2003)
T6 Human insulin	1MSO	PCF	Temp	(Smith, Pangborn et al. 2003)

34   *Table 1. Macromolecules whose structures were solved by crystals grown in microgravity, or whose resolution significantly improved.*

Company	Contact Information
The Aerospace Corporation	www.aero.org
Astrium North America	www.astrium-na.com
Astrotech Corporation	www.astrotechcorp.com
Aurora Flight Sciences	www.aurora.aero
Bionetics Corporation	www.bionetics.com
Bioserve	www.colorado.edu/engineering/BioServe
Boeing	www.boeing.com
CSS-Dynamac	www.css.dynamac.com
Hamilton Sundstrand	www.hamiltonsundstrand.com
Jamss America	www.jamssamerica.com
Kentucky Space, LLC	www.kentuckyspace.com
MDA	www.mdacorporation.com
MEI Technologies	www.meitechinc.com
Nanoracks LLC	www.nanoracks.com
Orbital Technologies Corporation	www.orbitec.com
Paragon TEC	www.paragontec.net
Qinetiq	www.qinetiq-na.com
Space Systems Concepts, Inc.	www.space-concepts.com
Space Systems Research Corporation	www.spacesystemsresearch.com
Tec-Masters, Inc.	www.tecmasters.com
Techshot	www.techshot.com
Teledyne Brown Engineering, Inc.	www.tbe.com
Thales Alenia Space	www.thalesgroup.com/space
UAB	www.uab.edu/cbse
Wyle Integrated Science and Engineering	www.wyle.com
Zin Technologies	www.zin-tech.com

Table 2. Implementation Partners for Flight Experiments on the ISS.

Funding Opportunities

There are various avenues that can result in funding for research to be conducted on the ISS, and the source of funding often dictates the availability of launch opportunities. Generally, funding for macromolecular crystal growth-related research is awarded through NASA-sponsored NRA's, ISS National Laboratory awards through other government agencies, private commercial enterprise, nonprofit organizations, and research awards sponsored by the ISS International Partners. An investigator wanting to fly just a few proteins or other macromolecules should initiate conversations with the points of contact for individual flight investigations. If the investigator has chosen a particular hardware he/she would like to use, then the entity flying that hardware could be contacted (NASA, CASIS, ESA, NASDA, Roscosmos).

It is not the responsibility of a researcher awarded an ISS flight experiment to fund costs associated with launch or the ISS laboratory facilities, although industrial entities may be asked to provide some funding to CASIS for their flights. Greater detail concerning current funding opportunities for ISS research can be found through the NASA ISS research website: http://www.nasa.gov/mission_pages/station/research/ops/research_information.html.

The NASA Solicitation and Proposed Integrated Review and Evaluation System (NSPIRES) can be accessed via http://nspires.nasaprs.com/external/.

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Acronyms

APCF	Advanced Protein Crystallization Facility
CGBA	Commercial Generic Bioprocessing Apparatus
COSIMA	Crystallization of Organic Substances in Microgravity for Applied Research
CRIM-M	Commercial Refrigerator Incubator Module – Modified
DCAM	Diffusion-controlled Crystallization Apparatus for Microgravity
EDCAM	Enhanced Diffusion-Controlled Crystallization Apparatus for Microgravity
EDR	European Drawer Rack
EGN	Enhanced Gaseous Nitrogen (EGN) Dewar
EM	Experiment module
ESA	European Space Agency
EXPRESS	EXpedite the PRocessing of Experiments to Space Station
GCB	Granada Crystallization Boxes
GCF	Granada Crystallization Facility
GLACIER	General Laboratory Active Cryogenic ISS Experiment Refrigerator
GN2	Gaseous Nitrogen Freezer
GN2	Gaseous Nitrogen-Dewar
HDPCG	High Density Protein Crystal Growth
IPU	Image Processing Unit
ISS	International Space Station
JAXA	Japan Aerospace Exploration Agency
LMM	Light Microscopy Module
MASER	Material Science Experiment Rocket
MERLIN	Microgravity Experiment Research Locker Incubator
MSG	Microgravity Science Glovebox
NASDA	National Space Development Agency of Japan
NIH	National Institutes of Health
NSPIRES	NASA Solicitation and Proposal Integrated Review and Evaluation System
OPCGA	Observable Protein Crystal Growth Apparatus
PCAM	Protein Crystallization Apparatus for Microgravity
PCDF	Protein Crystallization Diagnostics Facility
PCRF	Protein Crystallization Research Facility
PI	Principal Investigator
PromISS	Protein Microscope for the International Space Station
SCOF	Solution Crystallization Observation Facility
STES	Single-locker Thermal Enclosure System
STMV	Satellite Tobacco Mosaic Virus
VDA	Vapor Diffusion Apparatus

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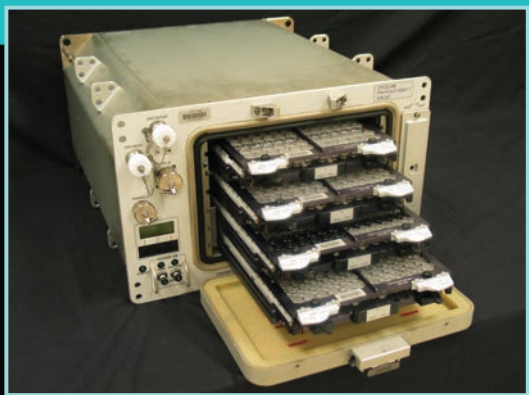http://www.nasa.gov/iss-science

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