SCM
UNEARTH THE POSSIBILITIES

MICROGRAVITY CRYSTALLIZATION CASE STUDIES

How biopharma is using research facilities in Space to solve terrestrial drug discovery and formulation challenges

Prepared by : Tanya Kanigan, PhD, Senior Market Analyst, SCM
March, 2025

SPACE COMMERCE MATTERS
spacecommatters.com

TABLE OF CONTENTS

INTRODUCTION: LEVERAGING MICROGRAVITY FOR PHARMACEUTICAL INNOVATION

Over the past several decades, researchers have investigated the effects of microgravity on crystallization, uncovering insights that have significantly advanced the development of molecular therapeutics. Since the early Space Shuttle and Spacelab missions of the 1980s, thousands of space-based crystallization experiments have consistently demonstrated that proteins, monoclonal antibodies, and small-molecule active pharmaceutical ingredients (APIs) form crystals with superior structural order in microgravity compared to Earth-based controls.

The microgravity environment eliminates sedimentation and suppresses convection, enabling solute molecules to diffuse slowly and uniformly. This promotes the growth of highly ordered, symmetric crystals with fewer defects, which enhances drug stability, solubility, and bioavailability. Notably, crystals grown in space often exhibit remarkable improvements in resolution when analyzed by X-ray or neutron diffraction, allowing for far more precise structural characterization.

These discoveries have had a direct impact on pharmaceutical research and development—particularly in structure-based drug design and formulation science. High-resolution protein structures derived from microgravity-grown crystals have enabled more accurate modeling of biomolecular interactions, enhancing early-stage drug discovery. Furthermore, the ability to control crystallization processes has informed the design of novel therapeutic formulations, including injectable and oral drugs with improved stability, manufacturability, and delivery profiles.

Recognizing these advantages, leading pharmaceutical companies—including Merck, Bristol Myers Squibb, and Eli Lilly—have invested in microgravity research through partnerships with NASA, the ISS National Lab, and commercial space organizations. These companies have launched experiments aboard the International Space Station (ISS) to refine protein crystallization techniques, optimize biologic formulations, and explore novel drug manufacturing processes.

In parallel, the emergence of commercial microgravity crystallization facilities (such as the Kirara high quality protein crystallization service) has expanded access, enabling smaller biopharma and academic researchers to conduct in molecular crystallization and formulation experiments in space. This integration of space-based research into both academic research and commercial drug development pipelines, has proven potential to accelerate drug discovery and enhance therapeutic performance.

THE SCIENCE OF MICROGRAVITY CRYSTALLIZATION: EFFECTS ON NUCLEATION AND CRYSTAL GROWTH

Crystallization is a fundamental process in materials science, structural biology, and pharmaceutical development, enabling the production of highly ordered molecular assemblies that are critical for both research and industrial applications. The quality of a crystal—determined by its size, uniformity, defect density, and diffraction properties—directly influences its utility in X-ray crystallography, drug formulation, and advanced materials. However, crystallization under normal gravity conditions is constrained by forces such as buoyancy-driven convection, sedimentation, and hydrostatic pressure gradients, which can introduce defects, non-uniform growth, and polymorphic variability.

Microgravity environments, such as those aboard orbiting space stations or free-flyer platforms, provide a unique setting in which these disruptive forces are significantly diminished. The absence of gravity-driven convection allows for more controlled mass transport, reducing structural imperfections and enabling the growth of larger, more ordered crystals. These effects have been particularly advantageous in pharmaceutical and protein crystallography applications, where high-quality crystals are essential for structural determination and drug formulation. This section reviews the fundamental principles of microgravity crystallization, including its effects on nucleation and crystal growth, the experimental methodologies used to leverage these conditions, and the broader scientific and industrial implications.

MICROGRAVITY EFFECTS ON NUCLEATION

Nucleation is the first step in crystallization, where solute molecules aggregate to form a stable crystal embryo. This process can occur through homogeneous nucleation, where crystals form spontaneously in solution, or heterogeneous nucleation, where they form on container walls or impurities. On Earth, heterogeneous nucleation dominates due to gravitational influences, leading to multiple, small, and often imperfectly formed crystals. Microgravity reduces the impact of container surfaces and enhances homogeneous nucleation, resulting in fewer but larger and more structurally ordered crystals (McPherson & DeLucas, 2015).

Another key factor in nucleation is mass transport. On Earth, convection continuously redistributes solute molecules, creating local fluctuations in supersaturation that can trigger premature nucleation events. This leads to numerous competing nuclei rather than the formation of a single well-structured crystal. In microgravity, where convection is absent, mass transport occurs primarily by diffusion, slowing the movement of solute molecules and reducing supersaturation fluctuations. As a result, nucleation occurs more homogeneously, producing fewer but higher-quality crystals with improved diffraction properties (Snell & Helliwell, 2005).

In addition to reducing convective mixing, microgravity also influences the stability of depletion zones—regions surrounding a forming crystal where solute concentration is lower than in the bulk solution. On Earth, these zones are frequently disrupted, leading to inconsistent crystal growth. In microgravity, depletion zones remain more stable, delaying secondary nucleation and promoting larger, more ordered crystal formation (Garcia-Ruiz et al., 2002).

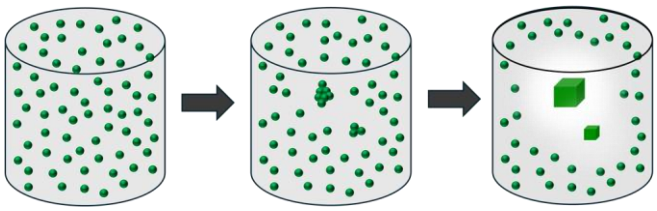


Figure 1 Graphical depiction of the nucleation of protein crystals and formation of a depletion zone around crystals growing in microgravity. (Figure adapted from: Japan Aerospace Exploration Agency (JAXA), "Protein Crystal Growth in Microgravity",

https://humans-in-space.jaxa.jp/protein/en/researchers/project/microgravity.html

THE SCIENCE OF MICROGRAVITY CRYSTALLIZATION: EFFECTS ON NUCLEATION AND CRYSTAL GROWTH

CRYSTAL GROWTH IN MICROGRAVITY

Once nucleation has occurred, crystal growth proceeds through the incorporation of solute molecules into the developing lattice. This process is highly sensitive to local solute concentrations, temperature gradients, and molecular diffusion. Under normal gravity conditions, convective currents disrupt the concentration gradients surrounding growing crystals, causing fluctuations in growth rates and an increased likelihood of secondary nucleation events. Additionally, sedimentation causes growing crystals to settle at the bottom of the container, where they may adhere to surfaces or cluster together, leading to irregular morphologies and crystal defects (Garcia-Ruiz et al., 2002).

In microgravity, these disruptive forces are eliminated, resulting in more controlled crystal growth. Solute molecules are transported primarily by diffusion rather than convection, leading to a stable supersaturation environment that enables more uniform molecular attachment to the crystal lattice (Snell & Helliwell, 2005, Martirosyan, A, 2022). This diffusion-limited growth mode has several key advantages:

- Reduced defect density: Under diffusion driven microgravity conditions, slower, more uniform solute transport favors the incorporation of correctly oriented solute molecules over impurities or mismatched species, which diffuse more slowly or inefficiently and are thus less likely to be included in the growing lattice.
- Larger, more symmetrical crystals: The absence of sedimentation prevents premature aggregation and clustering, allowing crystals to grow in free suspension.
- Improved diffraction properties: Crystals grown under microgravity have been shown to exhibit lower mosaicity and higher resolution X-ray diffraction, making them particularly valuable for protein crystallography and pharmaceutical applications.

MICROGRAVITY AND PROTEIN CRYSTALLIZATION

Microgravity is particularly beneficial for the crystallization of antibodies and other protein pharmaceuticals, where highly ordered lattices are essential for determining molecular structures and optimizing drug formulations. For instance, microgravity-grown monoclonal antibody crystals have been reported to exhibit superior structural integrity and stability, key factors for biopharmaceutical applications (Reichert et al., 2019). Additionally, experiments aboard the International Space Station (ISS) using counter-diffusion methods have shown that proteins such as lysozyme and thaumatin crystallize with greater uniformity and reduced mosaicity, a critical factor in X-ray diffraction analysis (Jackson et al., 2024).

A retrospective analysis of 353 protein crystallization experiments in microgravity revealed that microgravity-grown crystals exhibit improved morphology, larger volume, and lower mosaicity compared to their terrestrial counterparts (Jackson et al., 2024). The authors found that

72% of space-grown crystals were larger than their Earth-grown counterparts.

82% exhibited greater uniformity.

84% achieved improved diffraction resolution, with an average resolution enhancement of 0.30 Å.

77% had lower mosaicity, reducing structural imperfections

The findings reaffirm the value of space-based crystallization for improving protein structure determination, with over 90% of analyzed cases showing enhancement in at least one metric, reinforcing the case for continued investment in space-based crystallization research.

THE SCIENCE OF MICROGRAVITY CRYSTALLIZATION: EFFECTS ON NUCLEATION AND CRYSTAL GROWTH

IMPLICATIONS FOR DRUG DEVELOPMENT AND MANUFACTURING

To recap, the growth of crystals in microgravity is largely controlled by diffusion, which enables more precise molecular alignment and minimizes incorporation of impurities. Crystals remain suspended in solution, growing more symmetrically and avoiding interactions with surfaces that might introduce defects or distortions in the lattice structure

The ability to control nucleation and optimize crystal growth in microgravity has several implications for drug formulation, delivery, and manufacturing:

HIGHER-QUALITY PROTEIN CRYSTALS

Enables better X-ray diffraction for structure-based drug design (Snell & Helliwell, 2005).

IMPROVED DRUG STABILITY

Space-grown small-molecule APIs often have more stable polymorphic forms, reducing degradation risks (Bauser et al., 2024).

ENHANCED BIOAVAILABILITY

Optimized crystalline structures improve solubility and absorption, making formulations more effective (Reichert et al., 2019).

The case studies in this report highlight how microgravity crystallization research—spanning monoclonal antibody therapies, antiviral drugs, and small-molecule APIs—is driving new advancements that could reshape the future of pharmaceutical R&D.

PEMBROLIZUMAB (KEYTRUDA)

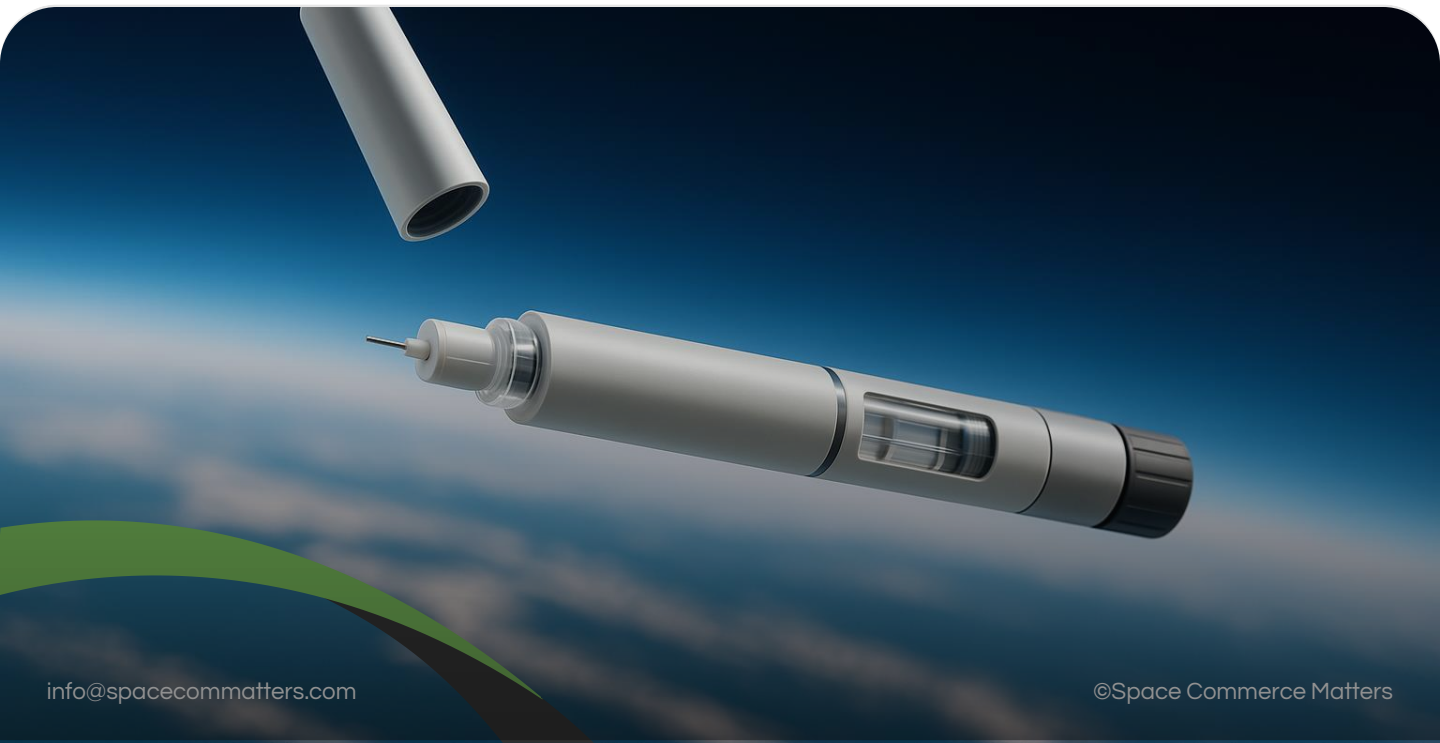
- Manufacturer: Merck & Co., Inc.
- Molecule Type: Monoclonal antibody (immune checkpoint inhibitor)
- Use: Pembrolizumab is used in cancer immunotherapy, targeting the PD-1/PD-L1 pathway to enhance the immune system's ability to recognize and destroy cancer cells.

BACKGROUND

Pembrolizumab, marketed as Keytruda, is one of the most significant advancements in cancer immunotherapy. As a humanized monoclonal antibody, it targets the PD-1 (programmed death receptor-1) on T-cells, a critical checkpoint in the immune system. By inhibiting the interaction between PD-1 and its ligands PD-L1 and PD-L2—proteins often overexpressed on tumour cells—Keytruda reactivates T-cells to recognize and destroy cancer cells. This mechanism has revolutionized the treatment of various cancers, particularly those with high PD-L1 expression, including melanoma, non-small cell lung cancer (NSCLC), and more.

Keytruda's impact on the oncology market has been profound. First approved by the FDA in 2014 for advanced melanoma, its indications have since expanded to over 20 different cancer types. By 2023, Keytruda had become a blockbuster drug, generating annual revenues exceeding $20 billion. The drug's success is due to its broad applicability across various cancer types and stages, including both metastatic and early-stage cancers. It has transformed the standard of care and solidified its position as a cornerstone therapy in Merck's oncology portfolio.

Despite its success, the drug's delivery via intravenous infusion presents challenges, driving research into alternative formulations that could improve patient convenience and compliance. Intravenous administration requires patients to visit healthcare facilities, which is time-consuming and often results in lost productivity. Additionally, it increases healthcare costs due to the need for specialized staff and equipment. Subcutaneous formulations could be administered in outpatient settings or even at home, potentially reducing costs and making the treatment process more convenient for patients.



PEMBROLIZUMAB (KEYTRUDA)

TERRESTRIAL CHALLENGES

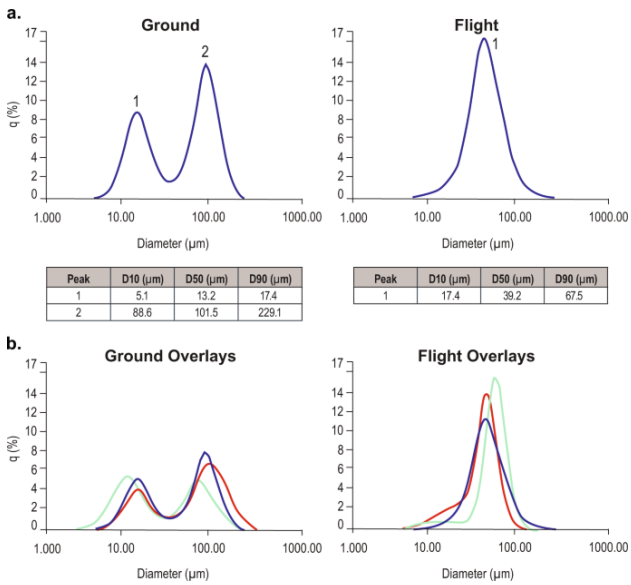
- Formulation: Developing stable and uniform crystalline preparations of monoclonal antibodies like pembrolizumab is particularly challenging due to their size and structural complexity. Pembrolizumab is currently administered to patients as an infusion, which can be time-consuming and inconvenient. A more patient-friendly formulation that could be administered as a subcutaneous injection requires the production of uniform crystalline suspensions with acceptable "syringeability" properties. A related challenge is developing a concentrated form of the antibody that is stable at room temperature to facilitate shipping and distribution.
- Purification: The purification of monoclonal antibodies like pembrolizumab typically involves multiple chromatography steps, which are time-consuming and costly. The ability to directly crystallize antibodies from the fermentation broth could streamline the purification process, reducing both time and costs.

MICROGRAVITY RESEARCH STUDIES

There is a long history of protein crystallization studies in low Earth orbit dating back to Space Lab experiments conducted in the 1980's (Wright, 2022). Merck, in collaboration with NASA, conducted protein crystallization research on 11 space shuttle missions, establishing the basis for subsequent work on the International Space Station National Lab (ISSNL). Four missions to the ISSNL have hosted research sponsored by Merck on Keytruda:

- SpaceX CRS-3 Mission (launch year 2014)
- SpaceX CRS-6 Mission (launch year 2015)
- SpaceX CRS-10 Mission (launch year 2017)
- SpaceX CRS-24 Mission (launch year 2021)

The research conducted during one of these missions, SpaceX-Commercial Resupply Services-10 mission has been described in detail in a peer reviewed journal article. In the study "Pembrolizumab Microgravity Crystallization Experimentation"(Reichert et al., 2019), Merck Research Laboratories explored the crystallization of pembrolizumab in microgravity. The research demonstrated that microgravity conditions facilitated the formation of highly uniform crystalline suspensions with a consistent particle size distribution. These suspensions were less viscous and more suitable for injectable formulations compared to ground-based controls.



Peak	D10 (µm)	D50 (µm)	D90 (µm)
1	5.1	13.2	17.4
2	88.6	101.5	229.1

Peak	D10 (µm)	D50 (µm)	D90 (µm)
1	17.4	39.2	67.5

Figure 2 Results from laser diffraction particle size analysis of samples crystallized on ground and in space. (Source: Reichert et al., 2019)

This pioneering work served as proof of concept, demonstrating that a microgravity crystallization experiment involving a full-length monoclonal antibody could produce a homogeneous crystalline suspension with reduced viscosity and improved rheological properties, as opposed to the bimodal crystalline suspension observed in Earth-based control experiments.

Dr. Paul Reichert, the Principal Investigator for the Keytruda microgravity studies, has also spoken about this research and its significance in conferences and webinars. A link to one of these presentations is provided in the references below (see Reichert, 2023).

PEMBROLIZUMAB (KEYTRUDA)

MICROGRAVITY RESEARCH IMPACT

- ### SUPERIOR PROTEIN FORMULATIONS:

The Merck pembrolizumab microgravity research showed that reduced sedimentation and convection currents in space allowed for better control over the crystallization process. This led to more uniform crystalline suspensions that were less viscous and exhibited more consistent syringeability properties than those produced on Earth.

- ### TRANSLATION TO TERRESTRIAL MANUFACTURING

Following the success of the on-orbit experiments, Merck applied the findings to improve their terrestrial manufacturing processes. Specifically, they incorporated techniques to replicate the benefits of microgravity on Earth, such as using rotational mixers to reduce sedimentation and temperature gradients to induce and control crystallization (Reichert et al., 2019). This approach enabled Merck to produce uniform crystalline suspensions on Earth with characteristics like those achieved in microgravity, including lower viscosity and improved syringeability properties. These improvements have facilitated the development of a subcutaneous injection formulation of Keytruda, potentially enhancing patient convenience and broadening its clinical use.

- ### DEVELOPMENT OF A NEW KEYTRUDA FORMULATION

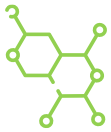
Merck has made significant progress in developing a subcutaneous injection formulation of Keytruda, leveraging the insights gained from microgravity research. Recent results from a Phase 3 clinical trial (Merck, 2024) indicate that this new formulation could match the efficacy of the intravenous form while offering a more convenient and less invasive administration method. If successful, this new formulation could further solidify Keytruda's position as a leading cancer therapy by improving patient adherence and quality of life, and by extending the critical patent life by many years.

FUTURE MISSIONS

As the International Space Station nears the end of its operational life, we expect that Merck's microgravity research on Keytruda and other drug molecules will transition to the new commercial space stations and "free-flyer" platforms currently under development. This shift will likely enable more extensive and cost-effective microgravity research, further advancing our understanding of crystallization and other critical processes in pharmaceutical development.

RITONAVIR (NORVIR)

- Manufacturer: Abbvie



- Molecule Type: Small molecule protease inhibitor
- Use: Ritonavir is an antiretroviral drug primarily used in HIV treatment. It is also commonly used in combination therapies to boost the efficacy of other protease inhibitors by inhibiting the enzyme CYP3A4, thereby slowing the metabolism of co-administered drugs.

BACKGROUND

Small molecule drugs like ritonavir can exist in multiple polymorphic forms—different crystal structures of the same molecule. These polymorphs can differ significantly in their physical and chemical properties, including solubility, bioavailability, and stability (Lee et al., 2011). Ritonavir was initially introduced to the market in 1996 by Abbott Pharmaceuticals as a capsule containing a crystalline form of the drug known as Form I. This product was believed to be stable and did not require refrigeration.

However, in 1998, a more stable but less soluble polymorph, Form II, was found to have contaminated the Abbott ritonavir gelcap product leading to failures in quality control (Bauer et al., 2001; Chemburkar et al., 2000). Form II had significantly lower bioavailability compared to Form I, which led to compromised therapeutic efficacy when taken orally. The unexpected emergence of Form II presented a major challenge, as even small amounts of Form II could induce the conversion of Form I into the less bioavailable polymorph. This issue ultimately halted the production of ritonavir capsules, forcing Abbott to withdraw the product temporarily, resulting in substantial financial losses estimated at over $250 million.

In response to this crisis, Abbott developed a refrigerated gelcap formulation and later introduced a tablet formulation of lopinavir/ritonavir (Kaletra) that did not require refrigeration (Klein et al., 2005). Advances in manufacturing allowed ritonavir tablets to be produced using a solid dispersion technique by melt-extrusion. This method ensured the stable production of Form I, allowing the product to be reintroduced commercially in 2010 (Hull & Montaner, 2011).

Research efforts to discover new polymorphs of ritonavir have continued, aiming to improve drug stability, solubility, and manufacturing processes. In 2022, two independent research groups concurrently discovered a new polymorph of ritonavir, designated as Form III (Bauser et al., 2024, Parent et al., 2023). One team, comprising researchers from Improved Pharma LLC and Varda Space Industries, identified Form III through melt crystallization techniques. Simultaneously, a separate group from AbbVie also reported the discovery of this polymorph through advanced crystallization methods, further demonstrating the importance of this line of research.

RITONAVIR (NORVIR)

TERRESTRIAL CHALLENGES

Producing stable forms of metastable drug polymorphs is inherently challenging due to the influence of gravitational forces, temperature gradients, and convection currents on Earth. For ritonavir, the ability to maintain metastable forms, such as Form III, without conversion to more stable but less bioavailable forms remains a key focus for pharmaceutical researchers. Advanced techniques, such as melt-extrusion and solid dispersion, are employed to address these challenges, but these methods often require significant optimization.

MICROGRAVITY RESEARCH STUDIES

The unique environment of microgravity offers distinct advantages for studying crystallization processes. Varda Space Industries, in collaboration with Improved Pharma LLC, recently conducted groundbreaking research on ritonavir crystallization aboard their W-1 mission.

- **MISSIONS**

The recent Winnebago-1 (W-1) mission by Varda Space Industries successfully returned ritonavir samples processed in space, which then underwent detailed post-flight analysis to assess the crystallization outcomes compared to Earth-based controls.

- **PUBLICATION**

The study "Return of the Ritonavir: A Study on the Stability of Pharmaceuticals Processed in Orbit and Returned to Earth" investigated the crystallization of ritonavir in microgravity aboard a compact unmanned capsule with Earth return capabilities (Bauser et al., 2024). The researchers used precise thermal control to crystallize ritonavir from its melt, aiming to produce the metastable Form III from Form II. The experiment successfully produced the metastable Form III of ritonavir, which is difficult to maintain on Earth due to its tendency to convert to more stable forms. The study confirmed that the microgravity environment allowed for precise control of the crystallization process, resulting in stable crystals that remained intact even after re-entry to Earth.

Figure 3 The landing of Varda's W-1 capsule in Australia after a successful 1st mission. (Source Varda Space Industries press kit)

- **PATENT APPLICATION**

VARDA Space Industries filed an international patent application (WO2024/000123) entitled "Processes for preparing solid state forms" on Aug 31, 2024 further demonstrating the commercial potential of these innovations.

RITONAVIR (NORVIR)

MICROGRAVITY RESEARCH IMPACT

Advancing Small Molecule Polymorph Research: While the benefits of microgravity for protein crystallization have been well-documented (Jackson et al., 2024), relatively few microgravity studies have involved organic small molecules. The successful production of the metastable Form III of ritonavir in low Earth orbit represents one of the first significant efforts to investigate potential for space-based research to overcome terrestrial challenges in small molecule drug development. The findings demonstrate that microgravity can provide control over crystallization, enabling the production of metastable forms that are difficult to achieve on Earth.

Increasing Pharmaceutical Industry Awareness for In-Space Drug Development and Manufacturing: The success of the Varda W-1 mission has garnered significant attention within the pharmaceutical industry. Varda and Improved Pharma LLC have presented their findings at prominent industry conferences, sparking interest in the broader application of space-based research for small molecule drug formulation.

ONGOING MISSIONS

On January 14, 2025, Varda's second mission, W-2, launched on a SpaceX racket and spent 6 weeks in orbit before landing at the Koonibba Test Range in South Australia on February 28. Varda has indicated in their press release that this payload includes "research that expands capabilities for in-space pharmaceutical processing" (Varda Space Industries, 2025).

HEMATOPOIETIC PROSTAGLANDIN D SYNTHASE (HPGDS)

- Manufacturer: N/A (Target for structure-based drug design)
- Molecule Type: Protein enzyme
- Use: Potential drug target for Duchenne muscular dystrophy (DMD) and other

BACKGROUND

Hematopoietic prostaglandin D synthase (HPGDS) is a sigma-class glutathione S-transferase that catalyzes the conversion of prostaglandin H2 to PGD2, a potent mediator of inflammation and neurological signaling. It is predominantly expressed in mast cells, macrophages, and antigen-presenting cells, playing a key role in allergic and inflammatory responses (Amselem et al., 2024).

Inhibiting HPGDS has emerged as a viable therapeutic approach for inflammatory and neurodegenerative diseases, as well as for slowing the progression of muscular dystrophy. H-PGDS inhibitors have shown potential in treating Duchenne muscular dystrophy by modulating the inflammatory environment associated with muscle degeneration (Komaki et al., 2020, Hamamura et al., 2024).

To develop effective inhibitors for a protein drug target, high-resolution structural data for that protein is essential. However, terrestrial crystallization experiments on H-PGDS have produced small, imperfect crystals with high mosaicity, limiting structural resolution. Recognizing that microgravity crystallization could produce superior protein crystals, researchers turned to space-based research facilities to improve crystal structure.

TERRESTRIAL CHALLENGES

01.

Crystallization Difficulties: The design of potent and selective HPGDS inhibitors relies on detailed X-ray crystallography structures that obtained from high-quality crystals using synchrotron x-ray sources. Conventional crystallization methods on Earth are limited by convection currents and sedimentation, leading to inconsistent crystal morphology and poor diffraction quality (Amselem et al., 2024).

02.

Structural Complexity: HPGDS exhibits dynamic conformational flexibility, with its active site undergoing structural changes during substrate binding and catalysis. This flexibility complicates the identification of stable binding pockets for inhibitors, as potential binding sites may shift or become occluded during the enzyme's catalytic cycle. Consequently, designing inhibitors with high affinity and selectivity requires detailed structural knowledge of all relevant conformational states (Cui et al., 2023).

HEMATOPOIETIC PROSTAGLANDIN D SYNTHASE (HPGDS)

IN-SPACE RESEARCH STUDIES

As previously discussed, microgravity offers a unique environment to grow more uniform and well-ordered protein crystals. The absence of buoyancy-driven convection and sedimentation allows crystals to form with fewer defects and enhanced diffraction properties. The first crystals structures of the HPGDS enzyme were produced from rat cDNA and crystallized by the hanging drop vapor diffusion method (Kanaoka et al., 1997). Even with synchrotron data the resolution was limited to 2.3Å, motivating researchers at the Osaka Bioscience Institute to participate in microgravity crystallization experiments to improve crystal size and quality.

MISSIONS

The Japan Aerospace Exploration Agency (JAXA, former NASDA led 9 missions to investigate HGPDS crystallization in space (Tanaka et al., 2011). The first of these was in 1997 on the space shuttle Atlantis (flight STS-84). The second mission samples were split between space shuttle Columbia and the Russian module of the ISS, but only the 2nd set of samples were successfully returned (Urade 2012). Subsequent experiments took place on the ISS as part of the JAXA Protein Crystal Growth (PCG) Project using multiple crystallization methods and experimental set-ups. A list of flights and microgravity crystallization "facilities" is provided in the table below.

FACILITY: GRANADA CRYSTALLIZATION BOX, LOCATED ON BOARD THE ISS RUSSIAN SERVICE MODULE		FACILITY: JAXA CRYSTALLIZATION BOX, LOCATED ON BOARD THE ISS RUSSIAN SERVICE MODULE		FACILITY: PROTEIN CRYSTALLIZATION FACILITY LOCATED ON BOARD THE ISS JAPANESE EXPERIMENT MODULE "KIBO"	
Flight	Launch Year	Flight	Launch Year	Flight	Launch Year
NASDA-GCF #1 NASDA-GCF #2 NASDA-GCF #3	2003 2003 2004	JAXA-NGCF #1 JAXA-NGCF #2 JAXA-NGCF #3	2007 2007 2008	Approx. 20 flights starting with JAXA PCG #1	2009-2024

HEMATOPOIETIC PROSTAGLANDIN D SYNTHASE (HPGDS)

KEY FINDINGS

MICROGRAVITY CRYSTALLIZATION INNOVATIONS AND PROCESS IMPROVEMENTS

Over the course of these missions JAXA, in partnership with academic researchers and the Japanese company Confocal Science Inc., improved the protein crystallization user experience by developing a comprehensive support system for technical and logistical challenges (Sato et al., 2006). They provided regular flight opportunities, user-friendly clerical support, and safety evaluations. To simplify space experiments, JAXA introduced innovative crystallization devices like the gel-tube method and the JAXA crystallization box, enabling effective counter-diffusion. They also developed ground-based tools for optimizing crystallization conditions and provided protocols for crystal harvesting and cryoprotection. These efforts made space experiments more accessible and significantly increased the success rate of obtaining high-quality crystals for structural biology research. In the final flight nearly 100% of the HGPDS samples were successfully crystallized in space (Urade et al., 2012)

ENHANCED CRYSTAL QUALITY

Space-grown HPGDS crystals were significantly larger and more uniform than their terrestrial counterparts, reducing lattice defects and mosaicity. According to a study by Tanaka et al., among 12 HPGDS crystals grown under different crystallization conditions, two exhibited better size and shape, while eight demonstrated enhanced resolution and reduced mosaicity and misalignment when cultivated in space compared to their counterparts grown on Earth under the same conditions (Tanaka, 2011).

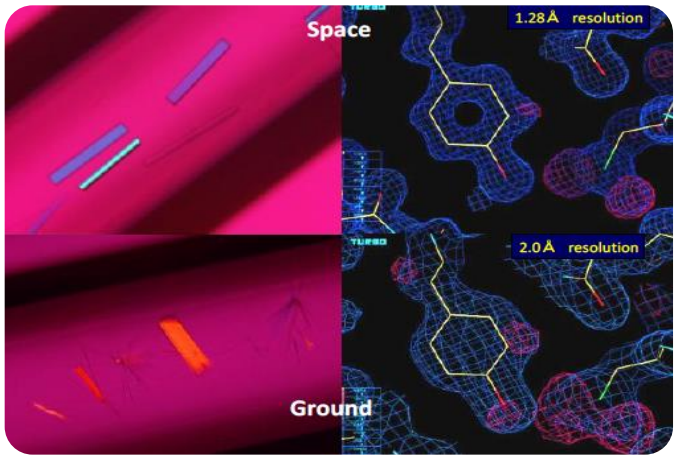


Figure 4 HPGDS-inhibitor complex crystals grown in space (upper left) and on Earth (lower left) are shown alongside their electron density maps, which have resolutions of 1.28 Å (upper right) and 2.0 Å (lower right), respectively. (Source: Urade et al., 2012)

IMPROVED X-RAY DIFFRACTION RESOLUTION AND ELECTRON DENSITY MAPS

Crystallization of HGPDS using a microgravity-based counter-diffusion method yielded crystals with markedly superior qualities compared to those grown under terrestrial conditions. The space-grown crystals of the human enzyme-inhibitor complexes diffracted to an impressive resolution of 0.95 Å (JAXA, 2021), whereas ground-grown crystals typically reached only about 1.3 Å—indicating a significant enhancement in atomic-level detail. In addition, the microgravity environment facilitated a substantial reduction in mosaicity, with average values dropping from approximately 0.7° for ground-grown crystals to about 0.3° for those produced in space (Takahashi et al., 2013). Crystals produced in the microgravity also yielded higher resolution electron density maps than crystals from ground control experiments. A representative example in Figure 4, shows that where the ground-grown crystals the benzene ring of the tyrosine residue at the enzyme's active site appears plate like at a resolution of 2.0 Å in ground-grown crystals, it appears donut shaped at a resolution of 1.28 Å in space-grown crystals (Urade et al., 2012).

HEMATOPOIETIC PROSTAGLANDIN D SYNTHASE (HPGDS)

MICROGRAVITY RESEARCH IMPACT

01.

Supporting Structure-Based Drug Design: The high-quality structural data obtained from microgravity-grown crystals allowed for a more precise understanding of the enzyme's active site and inhibitor binding mechanisms. This enabled structure-based drug design and optimization of lead compounds to inhibit enzyme activity (Cui et al., 2023; JAXA, 2021; NASA, 2024; Yamada, M et al, 2020).

02.

Enabling Development of Potential Treatments for DMD and Other Diseases: The high-resolution structural data obtained from these space-grown HPGDS crystals revealed detailed active site configurations and binding interactions that are crucial for rational drug design. These insights have enabled the development of potent HPGDS inhibitors targeting inflammatory diseases, allergies, and Duchenne muscular dystrophy (Komaki et al., 2020, Hamamura et al., 2024, Yamada, M et al, 2020). One of these inhibitors, TAS-205, manufactured by Taiho Pharma, is currently in Phase III clinical trials for Duchenne muscular dystrophy (ClinicalTrials.gov ID: NCT04587908) demonstrating the clinical potential of targeting HPGDS.

03.

Expanding Access to Microgravity Protein Crystallization Facilities: The hundreds of space crystallization experiments performed across many missions of the Protein Crystal Growth project led to improvements in not only crystal quality, but also to improved reliability, predictability, shorter time to answer, better user experience, higher throughput and lower cost per sample. Collaboration between Confocal Science, JAXA contractor Japan Manned Space Systems Corporation (JAMSS), and the ICE Cubes Service by Space Applications Services created the Kirara space manufacturing platform to facilitate commercial-scale crystallization in space and serve users worldwide.

As a result, microgravity protein crystallization facilities are no longer reserved exclusively for space researchers and big pharma. Non-space focused academic researchers, small biotech companies and students at all grade levels have sent samples to space for crystallization through the Kirara Service (JAMSS).

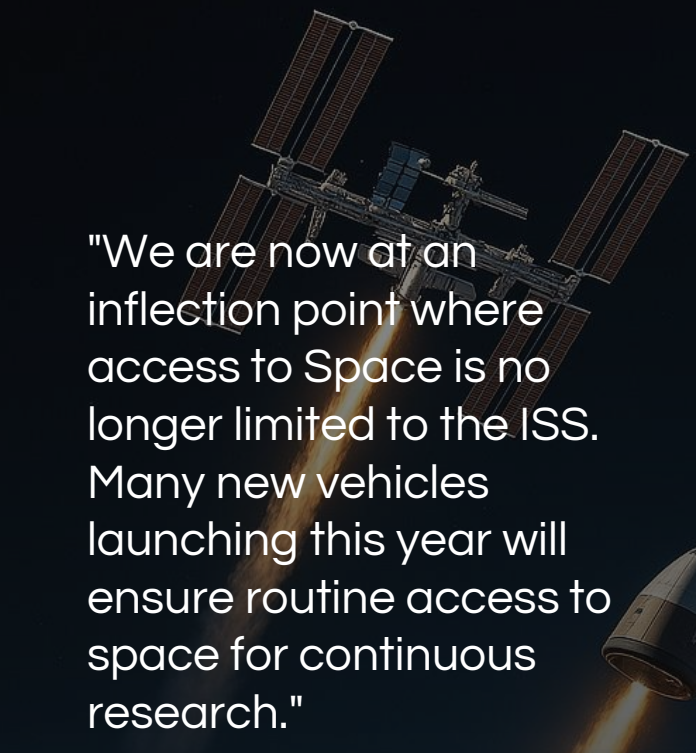
04.

Future Directions

- JAXA continues to support protein crystallization experiments on Kibo. They are also developing the "Low Earth Orbit Versatile Experimental System Technology," a semi-automated system that can support diverse microgravity research applications, including protein crystallization and cell studies, in the post-ISS world.
- The Kirara microgravity service continues its regular missions to the ISS and is accepting samples for its 2025 flights.

info@spacecommatters.com

ROUTINE ACCESS TO SPACE



"We are now at an inflection point where access to Space is no longer limited to the ISS. Many new vehicles launching this year will ensure routine access to space for continuous research."

4 PRIVATE SPACE STATIONS

2 will be in-orbit by late 2025 and the rest in the next years.

10 FREE FLYERS

Crewed and uncrewed vehicles, also launching this year, will enable missions in space lasting from 1 week to 2months.

14 RE-ENTRY CAPSULES

Some smaller capsules already in operation allow for a higher launch cadence in space.

ACKNOWLEDGEMENTS

The author would like to thank Dr. Hiroaki Tanaka of Confocal Science, Inc. and Dr. Miguel Ferreira of Space Applications Services for their valuable feedback and guidance in reviewing the HPGDS case study, and the introduction section.

For more information about new research opportunities in microgravity, please write to: info@spacecommatters.com

REFERENCES

- Aggarwal, M. D., Batra, A. K., Lal, R. B., Penn, B. G., & Frazier, D. O. (2011). *Growth and Characteristics of Bulk Single Crystals Grown from Solution on Earth and in Microgravity.* NASA Technical Reports Server (NTRS)
- Amselem, S., Kogan, D., Loboda, O., Levy, A., Feuchtwanger, Y., & Bavli, D. (2024). Monoclonal Antibodies from Space: Improved Crystallization Under Microgravity During Manufacturing in Orbit. *Journal of Exploratory Research in Pharmacology*, *9*(2), 96–105. doi: 10.14218/JERP.2023.00020
- Bauer, J., Spanton, S., Henry, R., Quick, J., Dziki, W., Porter, W., & Morris, J. (2001). Ritonavir: an extraordinary example of conformational polymorphism. *Pharmaceutical Research*, *18*(6), 859–866. doi: 10.1023/a:1011052932607
- Bauser, H. C., Smith, P. A., Parent, S. D., Chan, L. R., Bhavsar, A. S., Condon, K. H., … Radocea, A. (2024). *Return of the ritonavir: A study on the stability of pharmaceuticals processed in orbit and returned to earth*. doi: 10.26434/chemrxiv-2024-vb20g-v3
- Chemburkar, S. R., Bauer, J., Deming, K., Spiwek, H., Patel, K., Morris, J., … McFarland, K. (2000). Dealing with the Impact of Ritonavir Polymorphs on the Late Stages of Bulk Drug Process Development. *Organic Process Research & Development*, *4*(5), 413–417. doi: 10.1021/op000023y
- Cui, J., Feng, Y., Yang, T., Wang, X., & Tang, H. (2023). Computer-Aided Designing Peptide Inhibitors of Human Hematopoietic Prostaglandin D2 Synthase Combined Molecular Docking and Molecular Dynamics Simulation. Molecules, 28(15). doi: 10.3390/molecules28155933
- Garcia-Ruíz, J. M., Gonzalez-Ramirez, L. A., Gavira, J. A., & Otálora, F. (2002). Granada Crystallisation Box: a new device for protein crystallisation by counter-diffusion techniques. *Acta Crystallographica. Sect. D, Biological Crystallography*, *58*(Pt 10 Pt 1), 1638–1642. doi: 10.1107/s0907444902014464
- Hamamura, K., Yoshida, Y., Oyama, K., Li, J., Kawano, S., Inoue, K., … Aritake, K. (2024). Hematopoietic Prostaglandin D Synthase Is Increased in Mast Cells and Pericytes in Autopsy Myocardial Specimens from Patients with Duchenne Muscular Dystrophy. *International Journal of Molecular Sciences*, *25*(3). doi: 10.3390/ijms25031846
- Hull, M. W., & Montaner, J. S. G. (2011). Ritonavir-boosted protease inhibitors in HIV therapy. *Annals of Medicine*, *43*(5), 375–388. doi: 10.3109/07853890.2011.572905
- ISS National Lab (2018). Reshaping Drug Delivery with Protein Crystals. (n.d.). Retrieved August 31, 2024, from https://www.issnationallab.org/reshaping-drug-delivery-millions-of-crystals-at-a-time/
- Jackson, K., Hoff, R., Wright, H., Wilkinson, A., Brewer, F., Williams, A., … Wilson, A. M. (2024). An Analysis of Protein Crystals Grown under Microgravity Conditions. *Crystals*, 14(7), 652. doi: 10.3390/cryst14070652
- JAMSS: Kirara Crystallization Platform. Available at: https://www.jamss.co.jp/en/space_utilization/kirara/
- JAXA (2021). Drug Discovery to Reduce the Progression of Muscular Dystrophy by Targeting an Enzyme that Synthesizes Prostaglandin D2, Bioactive Fatty Acid | JAXA Human Spaceflight Technology Directorate. (n.d.). Retrieved March 6, 2025, from https://humans-in-space.jaxa.jp/en/biz-lab/news/detail/001832.html
- Kanaoka, Y., Ago, H., Inagaki, E., Nanayama, T., Miyano, M., Kikuno, R., … Hayaishi, O. (1997). Cloning and crystal structure of hematopoietic prostaglandin D synthase. *Cell*, *90*(6), 1085–1095. doi: 10.1016/s0092-8674(00)80374-8
- Klein, C. E., Chiu, Y.-L., Awni, W., Zhu, T., Heuser, R. S., Doan, T., … Hanna, G. J. (2007). The tablet formulation of lopinavir/ritonavir provides similar bioavailability to the soft-gelatin capsule formulation with less pharmacokinetic variability and diminished food effect. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *44*(4), 401–410. doi: 10.1097/QAI.0b013e31803133c5
- Komaki, H., Maegaki, Y., Matsumura, T., Shiraishi, K., Awano, H., Nakamura, A., … Takeda, S. (2020). Early phase 2 trial of TAS-205 in patients with Duchenne muscular dystrophy. *Annals of Clinical and Translational Neurology*, *7*(2), 181–190. doi: 10.1002/acn3.50978
- Martirosyan, A., Falke, S., McCombs, D., Cox, M., Radka, C. D., Knop, J., Betzel, C., & DeLucas, L. J. (2022). Tracing transport of protein aggregates in microgravity versus unit gravity crystallization. NPJ Microgravity, 8(1), 4. https://doi.org/10.1038/s41526-022-00191-x]
- McPherson, A., & DeLucas, L. J. (2015). Microgravity protein crystallization. *NPJ Microgravity*, *1*, 15010. doi: 10.1038/npjmgrav.2015.10
- Merck (2024). Merck Announces Phase 3 Trial of Subcutaneous Pembrolizumab With Berahyaluronidase Alfa Met Primary Endpoints | Business Wire. (n.d.). Retrieved March 3, 2025, from https://www.businesswire.com/news/home/20241119511079/en/Merck-Announces-Phase-3-Trial-of-Subcutaneous-Pembrolizumab-With-Berahyaluronidase-Alfa-Met-Primary-Endpoints
- Parent, S. D., Smith, P. A., Purcell, D. K., Smith, D. T., Bogdanowich-Knipp, S. J., Bhavsar, A. S., … Radocea, A. (2023). Ritonavir form III: A coincidental concurrent discovery. *Crystal Growth & Design*, 23(1), 320–325. doi: 10.1021/acs.cgd.2c01017
- Reichert, P., Prosise, W., Fischmann, T. O., Scapin, G., Narasimhan, C., Spinale, A., … Strickland, C. (2019). Pembrolizumab microgravity crystallization experimentation. *NPJ Microgravity*, *5*, 28. doi: 10.1038/s41526-019-0090-3
- Reichert, P. (2023). Conference presentation at the Forward Research & Innovation Summit: Dr. Paul Reichert: Microgravity Monoclonal Antibody Crystallization Experimentation - YouTube. (n.d.). Retrieved September 29, 2024, from https://www.youtube.com/watch?v=v9mpVolSXwI
- Sato, M., Tanaka, H., Inaka, K., Shinozaki, S., Yamanaka, A., Takahashi, S., … Tanaka, T. (2006). JAXA-GCF project - high-quality protein crystals grown under microgravity environment for better understanding of protein structure. *Microgravity - Science and Technology*, *18*(3–4), 184–189. doi: 10.1007/BF02870406
- Snell, E. H., & Helliwell, J. R. (2005). Macromolecular crystallization in microgravity. *Reports on Progress in Physics*, *68*(4), 799–853. doi: 10.1088/0034-4885/68/4/R02
- Takahashi, S., Tsurumura, T., Aritake, K., Furubayashi, N., Sato, M., Yamanaka, M., … Urade, Y. (2010). High-quality crystals of human haematopoietic prostaglandin D synthase with novel inhibitors. *Acta Crystallographica. Sect. F, Structural Biology and Crystallization Communications*, *66*(Pt 7), 846–850. doi: 10.1107/S1744309110020828
- Takahashi, S., Ohta, K., Furubayashi, N., Yan, B., Koga, M., Wada, Y., … Kamigaichi, S. (2013). JAXA protein crystallization in space: ongoing improvements for growing high-quality crystals. *Journal of Synchrotron Radiation*, *20*(Pt 6), 968–973. doi: 10.1107/S0909049513021596
- Tanaka, H., Tsurumura, T., Aritake, K., Furubayashi, N., Takahashi, S., Yamanaka, M., … Urade, Y. (2011). Improvement in the quality of hematopoietic prostaglandin D synthase crystals in a microgravity environment. *Journal of Synchrotron Radiation*, *18*(1), 88–91. doi: 10.1107/S0909049510037076
- Urade, Y., Nagata, N., Aritake, K. (2012). Crystallization of Hematopoietic Prostaglandin D synthase-inhibitor Complexes in Space and Development of Drugs used for Therapy of Duchenne Muscular Dystrophy, *J. Jpn. Soc. Microgravity Appl.*, 29(3). 125-131. Retrieved from https://www.jstage.jst.go.jp/article/jasma/29/3/29_125/_pdf and translated from Japanese to English using ChatGPT model o3-mini-high.
- Varda Space Industries (2025). W-2 • Varda Space Industries. (n.d.). Retrieved March 05, 2025, from https://www.varda.com/missions/w-2
- Wright, H., Williams, A., Wilkinson, A., Harper, L., Savin, K., & Wilson, A. M. (2022). An analysis of publicly available microgravity crystallization data: emergent themes across crystal types. Crystal Growth & Design, 22(12), 6849–6851. doi: 10.1021/acs.cgd.2c01056
- Yamada, M., Kihira, K., Iwata, M., Takahashi, S., Inaka, K., Tanaka, H., & Yoshizaki, I. (2020). Protein crystallization in space and its contribution to drug development. In Y. Pathak, M. Araújo dos Santos, & L. Zea (Eds.), *Handbook of space pharmaceuticals* (pp. 1–26). Springer International Publishing. https://doi.org/10.1007/978-3-319-50909-9_40-1