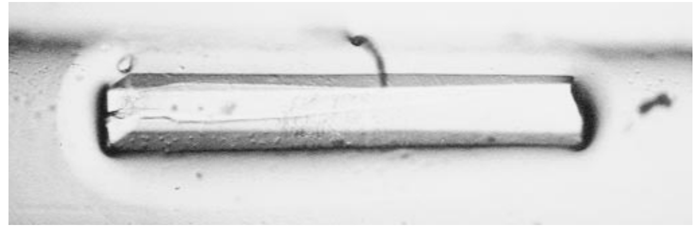
Protein Crystal Growth

Proteins play important and vital roles in our daily lives, from providing nourishment to fighting diseases. Because a protein's structure determines its function, expanding our knowledge of the structure of proteins has potential benefits to many areas of biotechnology. These benefits include new information on basic biological processes, development of food crops with higher protein content and increased resistance to disease, and basic research toward the development of more effective drugs.

The purpose of NASA's Protein Crystal Growth experiments is to produce large, well-ordered crystals of various proteins under controlled conditions in microgravity. These crystals will be used in ground-based studies to determine the three-dimensional molecular structure of each protein. One way to determine that structure is by using a process called *X-ray diffraction*, which requires large, well-ordered crystals. Protein crystals are very small — about the size of a grain of table salt — and sometimes take longer to grow in microgravity than on Earth. The microgravity environment minimizes sedimentation and the effects of convection on the crystal being grown. For many crystals, this results in a more uniform and highly-ordered structure with fewer defects than are found in the same proteins crystallized on Earth.

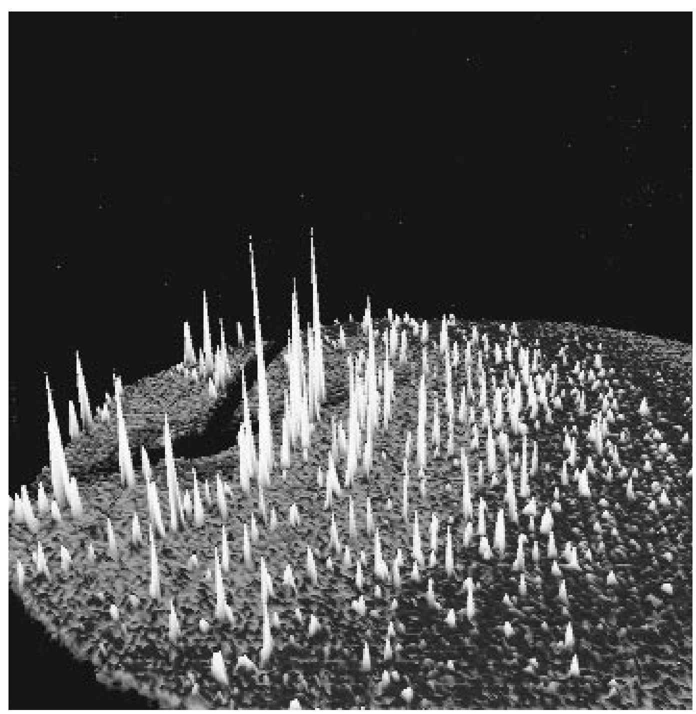
Through these experiments, scientists will continue to investigate how to control and optimize protein crystal growth in microgravity. From studying the growth rates — or *kinetics* — under differing conditions, investigators hope to improve the growth of protein crystals in microgravity, which may result in higher quality crystals and larger crystals of hard-to-grow proteins for structural studies. By identifying critical sites of a protein, researchers are able to affect the protein's behavior by altering the appropriate genetic components. By designing organic compounds that interact in a highly specific manner with selected areas of a protein, researchers may be able to create new pharmaceutical compounds or mimic the biological function of a protein using synthetic peptides.

The protein crystal growth experiments on the First United States Microgravity Laboratory were very successful. Of the more than 30 proteins flown, nearly half produced crystals large enough for X-ray diffraction studies. Canavalin, a plant protein, formed some of the largest crystals of this protein ever grown (more than 2.0 millimeters in length), as did Human Proline Isomerase, a protein associated with transplant rejection and a target of drug designers. The isomerase crystals were superior in symmetry, clarity, smoothness of surfaces, and definition of facets to those grown on Earth. They were also much larger than their Earth-grown counterparts and yielded the highest quality data ever collected. Large, well-formed crystals of proteins important to researchers studying immune disorders also were grown. These included HIV Reverse Transcriptase Complex, a protein important in the design of drugs that fight Acquired Immune Deficiency Syndrome. The three-dimensional structure of Factor D, a protein that plays a role in cardiovascular complications including inflammation after open-heart surgery, was completed using the high-quality crystals obtained on the first mission.



This crystal of the Factor D protein was grown on the USML-1 mission.

On this mission, two protein crystal growth experiments will be conducted along with companion experiments in the Glovebox. The Single-Locker Protein Crystal Growth experiment uses new and refined experiment trays to grow protein crystals in more than 300 chambers that are housed in a single-locker thermal enclosure system. The Commercial Protein Crystal Growth experiment occupies a Commercial Refrigerator/ Incubator Module and grows large quantities of crystals using a temperature change to start the process. All thermal enclosure systems will be installed in middeck lockers.



Using the high-quality crystals grown in microgravity, scientists use X-ray diffraction data, seen here in this visual display, to learn what atoms are present and how they are arranged.

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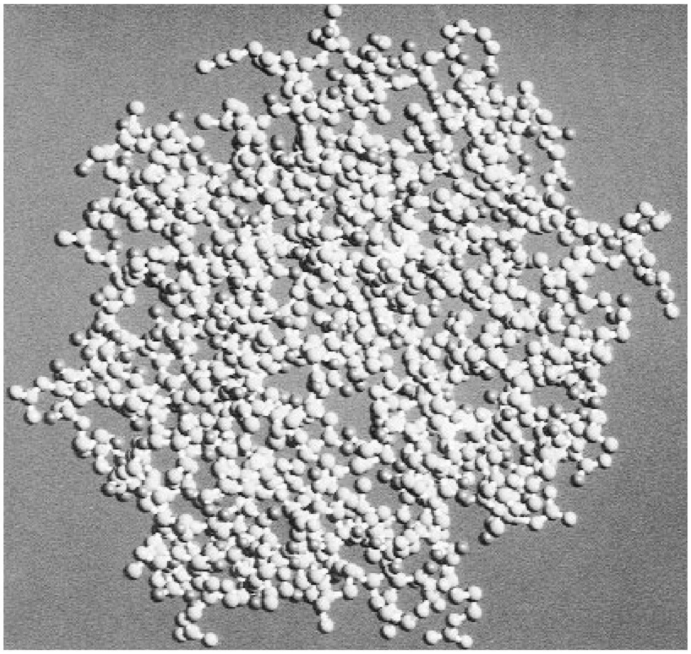

From the X-ray diffraction data, scientists can develop computer-generated 3-dimensional models of the proteins.

Single-Locker Protein Crystal Growth

Principal Investigator:
Dr. Daniel Carter, NASA Marshall Space Flight Center

Purpose: To grow small quantities of various proteins in a single-locker thermal enclosure system using the vapor diffusion method
Significance: The most often employed method of growing protein crystals is a technique that relies on water vapor pressure differences between two solutions within a chamber to create ideal growth conditions. This technique, called *vapor diffusion*, uses a chamber filled with a solution that attracts water — called a *precipitant* and composed usually of salts — surrounding a solution of water and proteins. Differences in vapor pressure between the two solutions causes water to diffuse out of the protein solution and into the surrounding reservoir. As the protein concentrations increase, crystals begin to nucleate and grow.

This experiment relies on two pieces of hardware, the Vapor Diffusion Apparatus trays and the Protein Crystallization Apparatus for Microgravity units. In the trays, the growth solutions are contained in double-barreled syringes; in the units, the growth solutions are on individual pedestals surrounded by circular chambers. Each tray has 24 growth chambers, while each unit has 7 chambers. On orbit, a crewmember uses a special tool on the trays that presses the plungers on each of the double-barreled syringes and mixes the solutions, forming a hanging drop at the end of the syringe in each chamber. The units are activated when a crewmember turns a knob that lifts a seal, exposing the protein solutions to the precipitating agents. Once activated, the trays and units are placed in the thermal enclosure and allowed to grow for the remainder of the mission. Reversing the procedure deactivates the experiments to protect the crystals during landing.

Commercial Protein Crystal Growth

Principal Investigator:
Dr. Larry DeLucas, Center for Macromolecular Crystallography, Birmingham, Alabama

Purpose: To grow large quantities of crystals of various proteins using the batch process method
Significance: In the Protein Crystallization Facility, carried in a Commercial Refrigeration/ Incubation Module, a temperature change initiates the crystallization process. The facility contains four cylindrical crystallization containers that are kept at 40 °C until the Shuttle reaches orbit; at that time, they are gradually cooled to 22 °C over a 24-hour period. Crystals form as the chamber cools, and crystal growth continues at 22 °C for the remainder of the mission. The experiment also will grow additional crystals in a Vapor Diffusion Apparatus located in 4 °C Commercial Refrigeration/ Incubation Module.