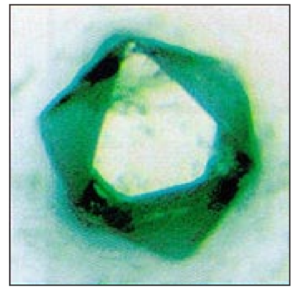
# Mir Protein Crystal Growth Program Nearing Conclusion

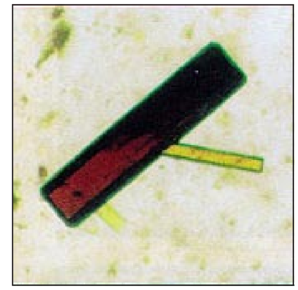
## *Space Shuttle-Mir Science Program (STS-89/STS-91)*

Biotechnology Program Office

Protein Crystal Growth Program

**Hardware**
**$LN_2$ Dewar**


APO ferritin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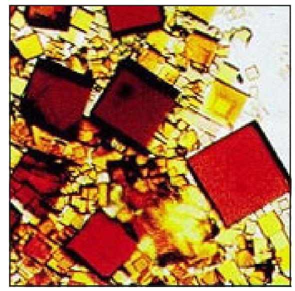
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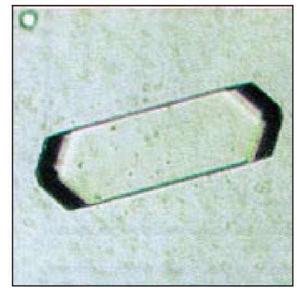
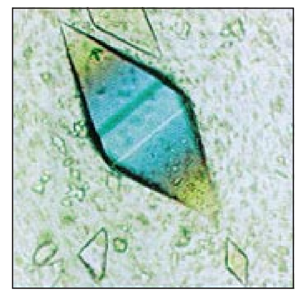

Satellite tobacco mosaic virus


Thaumatin


Rhombohedral canavalin

A highly succesful program to develop a new method of preparing large quantities of protein crystals goes into the home stretch on the STS-89 Space Shuttle mission to Russia's space station Mir. Since June 1995, NASA has delivered almost 3,000 specimens to thaw and then crystallize over a period of several months aboard Mir. Space Shuttle Atlantis will deliver the seventh and last dewar in late January, and retrieve it on the STS-91 mission in May when the Space Shuttle-*Mir* Science Program formally ends its flight phase.

Results from the first sets of Dewar samples show that the flash-frozen liquid-liquid diffusion and flash-frozen batch methods of preparing protein crystals is valid. Results from the first dewars have been incorporated into subsequent experiments (detailed analysis of the first six Dewars of samples have shown highly promising results).

Proteins are important, complex biological molecules which serve a variety of functions in all living organisms. Determining their molecular structure will lead to greater understanding of their functions in living organisms. Many proteins can be grown as crystals and their molecular structures determined through analysis of the crystals by X-ray crystallography. Unfortunately, some crystals grown in the 1-g environment of Earth often have internal defects that make such analysis difficult or impossible.

As demonstrated on U.S. Space Shuttle missions since 1985, certain protein crystals grown in space are larger, have fewer defects and have greater internal order, than their Earth-grown counterparts. Extended duration in orbit is especially valuable in protein crystal growth (PCG). The Space Shuttle-*Mir* Science Program provides the opportunity to grow protein crystals for longer periods than the U.S. Shuttle can now provide. The months of growth time on *Mir* will help shape investigations planned for the International Space Station.

One immediate purpose of these experiments is to obtain large crystals of sufficient size and quality to compare with corresponding crystals grown in Earth-based laboratories. An ongoing objective is to determine the best mixtures to use in flash-frozen batch and liquid-liquid diffusion techniques for growing protein and virus crystals on long-duration space missions.

*Mir* comprises a core module plus research modules, and supports work in life and microgravity sciences. To provide an early understanding of operations aboard International Space Station, NASA and RSA are conducting a series of U.S. missions to *Mir.* The PCG Dewar program has not been affected by the difficulties in Mir's Spektr module. The Dewars are placed in the Kvant module and, in the event, none was aboard Mir when the accident occured.

## Protein crystal growth

PCG experiments aboard *Mir* use the liquid-liquid diffusion method and the batch method. Liquid-liquid diffusion allows the precipitant to move through the solution and induces the proteins to crystallize as they meet. A growing crystal takes protein from the immediate vicinity. More protein then diffuses into the area, slowly, allowing formation of large crystals with highly uniform internal order essential for x-ray diffraction analysis of crystals after the mission. The frozen batch process sets conditions for crystallization as soon as the mixture thaws. The samples are contained in Tygon plastic tubing, heat-sealed at each end, or in capped Nunc cryogenic storage tubes. Sample sizes range from about 5 to 5,000 microliters (5 µl to 5 ml). The solutions are loaded, then flash frozen in liquid nitrogen at -196 ˚C to block diffusion and crystal growth until thawing occurs on *Mir*.

**Marshall Space Flight Center**
**Huntsville, Alabama**

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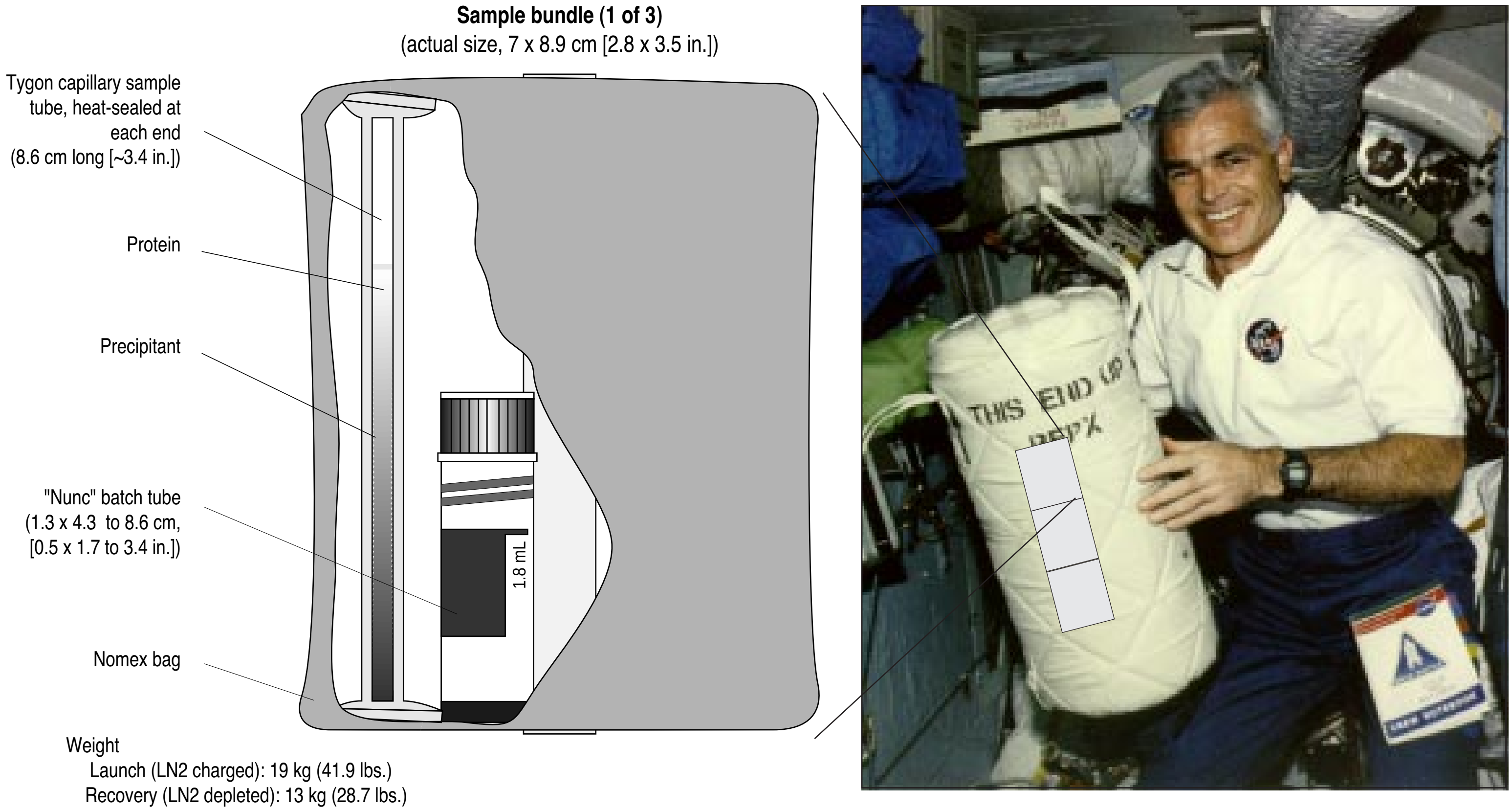


## Flight hardware

Equipment used in the *Mir* PCG experiments is simple and requires no crew activity. Each Dewar includes three sample bundles, an insert, a nitrogen Dewar, and a cover bag. A total of 250 to 500 samples is carried in each Dewar, depending on the mix of tubes and sizes. Three sample bundles are stacked in an aluminum cylinder insert, 8.9 cm (3.5 in.) in diameter and 34.3 cm (13.5 in.) long.

The aluminum Dewar, 24 cm (9.5 in.) in diameter and 50.8 cm (20 in.) tall, has a calcium silicate absorbent which is filled with liquid nitrogen and surrounded by a vacuum insulating jacket. It is sealed with a urethane foam neck plug and capped. The Dewar is carried in a Nomex bag for easy handling.

## Flight activities

The Dewar is a passive device with no active refrigeration. It warms slowly as its liquid nitrogen, which was loaded before launch, boils off. After the Shuttle docks with *Mir*, the crew secures the Dewar in a quiet area of *Mir* to minimize vibration (as shown above). The Dewar's liquid nitrogen supply boils off into *Mir*'s oxygen/nitrogen atmosphere, and the samples thaw after the nitrogen evaporates (over a period of 10 days). This ensures that crystallization occurs in the microgravity of space. The Dewar and its contents reach cabin temperature, 22 ˚C, and crystallization takes place over the next few months. The Dewar will remain at 22 ˚C, ±4 ˚C, through retrieval shortly after the Shuttle landing and the return of samples to the investigators.

## Candidate samples

An initial examination of the first batches of flash-frozen liquid-liquid diffusion samples grown aboard *Mir* shows that a number of uniform, high quality crystals were grown. So far, the best crystals have been in relatively long tubes with small diameters. This supports the hypothesis that a long, thin diffusion cell is the optimal design for liqud-liquid diffusion growth. Variations in temperature, vibration, and microgravity levels do not appear to impair the growth of the crystals. In the first Dewar, a gradient in size and quality was observed from one end of the tubes to the other. Thus the samples contain a natural variation along the length of the tube which can provide additional information on the ideal crystal growth conditions. Using small volumes lets scientists screen large numbers of experimental conditions. Up to 15,000 samples in small tubes could be carried in a single Dewar.

The samples were selected by a NASA selection committee. The lead investigator is Dr. Alexander McPherson of the University of California, Riverside. The research associate is Dr. Stan Koszelak, also of the University of California, Riverside.

## Notes