

PROTEIN CRYSTAL GROWTH
(5-IML-1)

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Proteins (enzymes, hormones, immunoglobulins, and numerous other types) account for 50% or more of the dry weight of most living systems and play a crucial role in virtually all biological processes. Since the specific functions of essentially all biological molecules are determined by their three-dimensional structures, it is obvious that a detailed understanding of the structural makeup of a protein is essential to any systematic research pertaining to it. At the present time, protein crystallography has no substitute: It is the only technique available for elucidating the atomic arrangements within complicated biological molecules.

Most macromolecules are extremely difficult to crystallize, and many otherwise exciting and promising projects have terminated at the crystal growth stage. Single crystals that have dimensions of 0.2-1.0 mm on a side are generally required for x-ray crystallographic analyses of macromolecular structures, and much larger crystals are required for neutron diffraction analyses. Proteins and other biological macromolecules often yield small micro-crystals readily, but it might then take several years of trial and error experimentation before these micro-crystals can be induced to grow large enough for a complete structural analysis. Even when large crystals are obtained, the crystals of essentially all biological macromolecules diffract rather poorly due to internal disorder. Thus, there is a pressing need to better understand protein crystal growth, and to develop new techniques that can be used to enhance the size and quality of protein crystals.

In principle, there are several aspects of microgravity that might be exploited to enhance protein crystal growth. According to theoretical considerations and experiment results, the major factor that might be expected to alter crystal growth processes in space is the elimination of density-driven convective flow. Convection in solution growth is caused by density gradients that occur when solute is depleted from the solution at the growing crystal surfaces. The density-dependent convection might be expected to affect protein crystal growth from aqueous solutions in several different ways. Convection will force solution to flow past the crystal, thus bringing material to the growing crystal surfaces at a rate that is significantly different from the steady-state diffusion rate that would be predominant in quiescent solutions. The flow patterns may generate significant variation in concentration at different parts of a crystal, thus leading to non-uniform growth rates. Also, convection may lead to significant physical stirring of growth solutions; in general, it is expected that such stirring effects might alter nucleation in growth processes.

Another factor that can be readily controlled in the absence of gravity is the sedimentation of growing crystals in a gravitational field. When a protein crystal grows from

aqueous solution on Earth, it generally migrates to the top or the bottom of the crystallization vessel (depending on whether its density is greater or less than the density of the solution). Therefore, protein crystals often grow from solution at an interface where all sides of the crystal are not equally accessible to the crystallizing solution. (In most cases, sedimentation causes proteins to crystallize as fused masses that contain highly disordered crystalline arrays.) Under microgravity conditions, it is expected that protein crystals will not display this tendency to migrate away from initial nucleation sites, and can thus grow in isotonic environments, forming discrete, independent nucleation sites.

Another potential advantage of microgravity for protein crystal growth is the option of doing containerless crystal growth. Contacts with vessel walls often lead to heterogeneous nucleation in crystal growth solutions. In the microgravity environment, it may be possible to form stable spherical droplets of crystallizing materials, which might be suspended by acoustical levitation or other methods. It is definitely possible to form relatively large stable droplets of protein solutions by extruding solutions from a pipette or a syringe; thus, protein crystals might be grown under microgravity conditions in relative large droplets adhering to syringe tips, without the extensive wall effects that generally accompany crystallization experiments on Earth.

As a result of the above theories and facts, one can readily understand why the microgravity environment established by Earth-orbiting vehicles is perceived to offer unique opportunities for the protein crystallographer. This perception led to the establishment of the Protein Crystal Growth in a Microgravity Environment (PCG/ME) project that continues today under NASA sponsorship. This project has advanced from simple hand-held devices (containing only a few protein solutions) to a more complex system involving 60 or more individual protein experiments in a thermally conditioned environment. The results of experiments already performed during STS missions have in many cases resulted in protein crystals being grown that are significantly larger and more structurally correct than the best specimens produced on Earth. Thusly, the near-term objective of the PCG/ME project is to continue to improve the techniques, procedures, and hardware systems used to grow protein crystals in Earth orbit. A large number of industrial guest investigators and co-investigators are involved in the project (reference Attachment A) and multiple flight opportunities are obviously required to accomplish the referenced objectives.

The hardware complement currently in use consists of a Refrigerator/Incubator Module (R/IM) that provides protection and a controlled thermal environment (Figure 1), a Vapor Diffusion Apparatus (VDA) designed to manipulate and process the protein samples (reference Fig. 2). The VDA tray consists of 20 chamber assemblies, gang-operated dual cylinder syringes, and gang-operated plug mechanisms. The overall assembly is mounted between two aluminum/Plexiglas windows with a soft silicon rubber (35 to 65 durometer) gaskets providing sealing of the overall assembly to prevent external leakage and chamber to chamber leakage.

The dual-cylinder syringe is made of polysulfone and is operated by a ganged piston assembly. Each cylinder of the syringe contains a maximum of 40 μ l of either precipitant or protein solution. When the wheel that operates the ganged pistons is rotated, the solutions are

either forced onto the syringe tip or withdrawn into the syringe cylinders, depending upon the direction of the rotation. The drive assembly is designed such that from the launch configuration of the syringe piston, eight to nine revolutions of the wheel are required to push solutions in the cylinder to the tip of the syringe to form a drop. Several peripheral items of equipment such as a temperature logging device and photographic equipment are also utilized. The current system was first flown on STS-26 in September 1988, and was subsequently reflown on STS-29 in April 1989.

The STS-26 orbiter middeck experiment was the first temperature controlled or systematic investigation to grow useful crystals by vapor diffusion in microgravity. Results from this experiment were very encouraging, since high quality crystals were obtained from most of the protein and enzyme samples flown and four samples grew crystals of exceptional size and quality. The STS-29 results were not quite as promising as those from STS-26 due to several hardware/procedural problems. However, crystals of greater size and uniformity than those grown on Earth were still produced. The knowledge gleaned from these flights is being applied to follow-on PCG flights, in order to help define the flight and post-flight techniques and analytical methods and procedures used.

The IML-1 mission will accommodate two R/IMs (4 °C and 22 °C). (Each R/IM replaces one standard middeck locker, and uses Orbiter-provided 28 Vdc power from a standard outlet in the ceiling of the middeck.) The samples being grown will utilize seeding crystals in the growth process. The seeding crystal is inserted through a small opening in the top of the VDA window using a drop dispenser. The drop dispensers are stowed in the R/IM during the mission. The seed crystal is contained in a drop of stabilizing solution that is planted by allowing the stabilizing solution drop to come into contact with the protein drop. The PCG experiment is nominally activated during flight Day 1 activities. During activation, the PCG photo TV setup is performed, the R/IM door is removed, and the solutions in the syringes are mixed by attaching a handwheel to a syringe piston ganging mechanism and rotating it alternately clockwise and counter-clockwise and then again clockwise to deploy the droplets from the tips of the syringes. Once the droplets are deployed, the PCG photo session begins. During the photo sessions, a 35 mm camera is used to photograph the droplets on the tips of the syringes. The seed crystals are planted 12 to 36 hours after activation. After completion of the seeding session the PCG unit is left untouched until deactivation in the R/IM.

Disturbances during critical times such as activation plus 12 hours to activation plus 36 hours could cause premature nucleation. These disturbances could be caused by treadmill activity or RCS firings. A showering effect results when crystal formation is disturbed, thus resulting in many little crystals. These vibrational forces should be kept to a minimum for maximum scientific results from the experiment.

PCG is deactivated on the last flight day prior to deorbit, at the latest possible time. The deactivation process involves completing the final photo session, which is taking photographs of the crystals formed on the tips of the syringes. Once the photo session is completed, the droplets

are retracted back into the syringes, the R/IM door is closed, and the PCG unit is ready to return to the landing site.

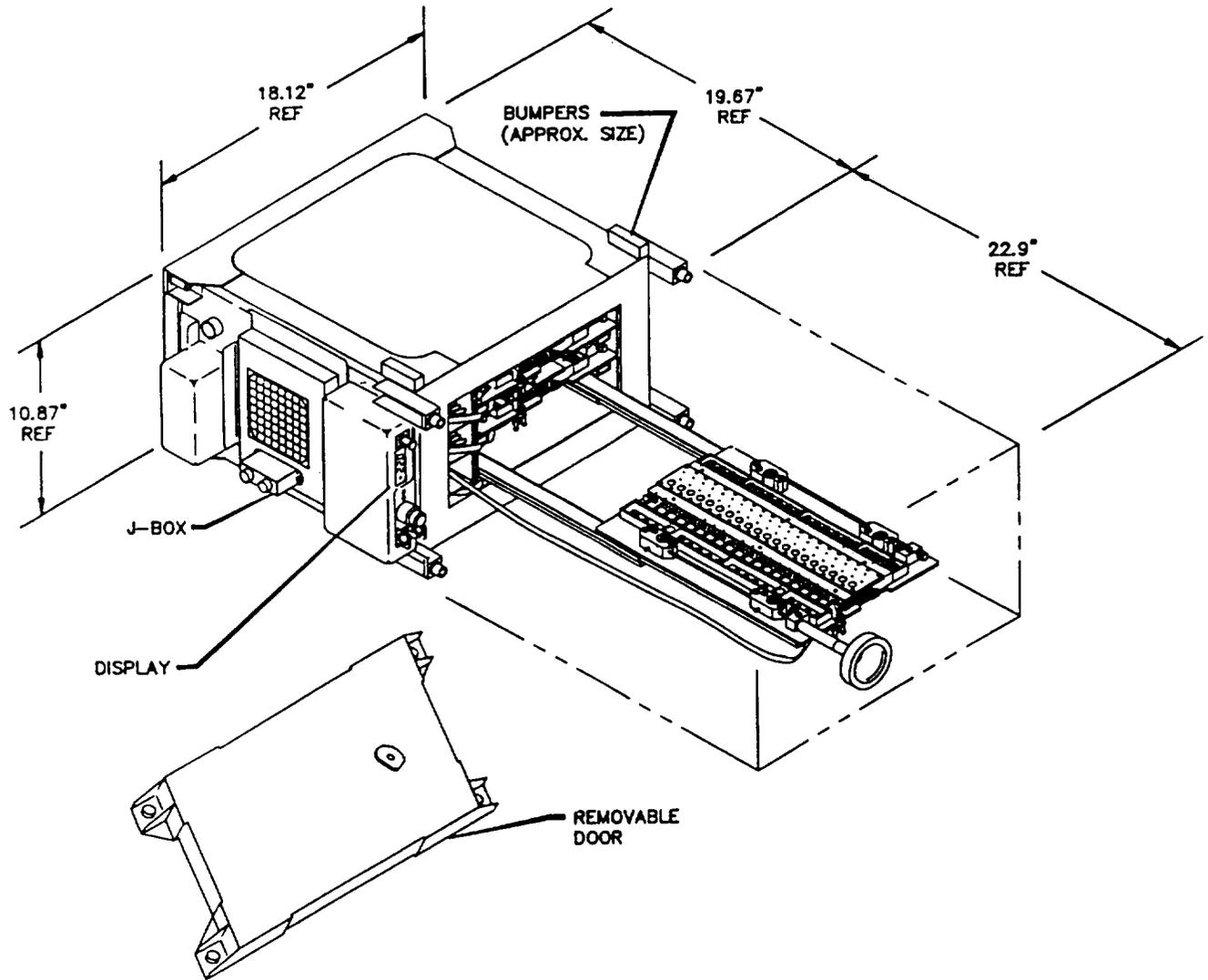


Figure 1. Refrigerator/Incubator Module (R/IM).

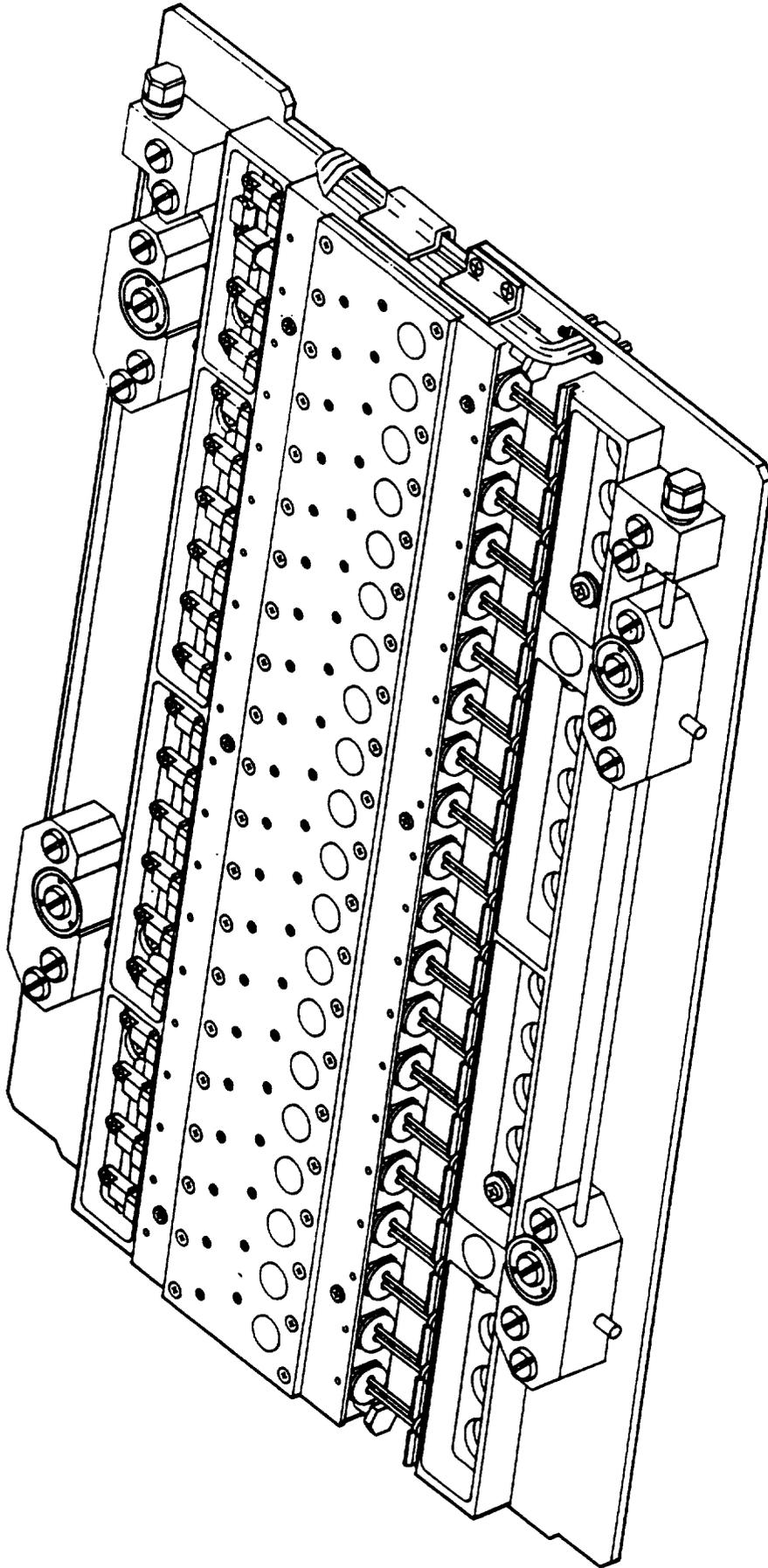


Figure 2. Vapor Diffusion Apparatus (VDA).