ADVANCED PROTEIN CRYSTALLIZATION FACILITY (APCF)

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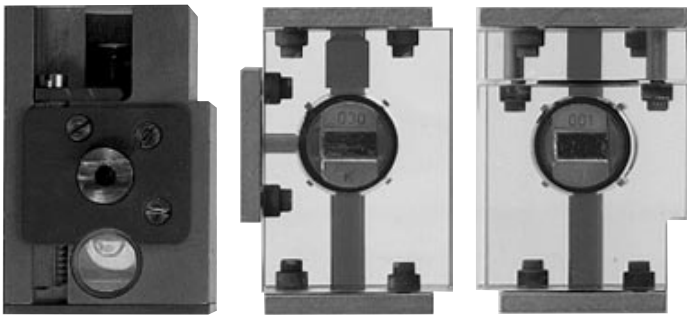

A *B* *C*

The APCF is the first facility in which protein crystals can be grown by three techniques: (A) hanging drop method (B) free interface diffusion (C) dialysis

Conditions on Earth limit the size and quality of protein crystals, but the microgravity environment of space is expected to allow the manufacture of larger, more highly ordered crystals. The APCF is the first facility ever designed to use three methods of protein crystal growth: liquid-liquid diffusion, or free interface diffusion, in which a protein solution and a salt solution are separated by a buffer and are allowed to mix together slowly once the Shuttle is in orbit; dialysis, with protein and salt solutions separated by a membrane; and vapor diffusion, or the hanging drop method, where crystals form inside a drop of protein solution as solvent from the drop diffuses to a reservoir. For all three methods, crystallization will occur at a constant temperature of 20 °C.

Video images will be made of crystals as they form. After the mission, the images will allow investigators to study the history of crystal development in microgravity. Scientists are interested particularly in why and how crystals nucleate to begin crystal formation. When the crystals return from space they will be analyzed, using precision X-ray beams (synchrotron radiation, whenever available), sophisticated detectors, and data processing equipment to determine the internal arrangement of their molecules. As X-rays diffract off the atoms of the crystals, a computer will map each atom's position. With these maps of larger, more highly ordered crystals, scientists may be able to expand our understanding of biological processes on the molecular level, which could lead to applications in medicine and agriculture.

Crystallization of Apocrustacyanin C

**Principal Investigator:
Dr. Naomi Chayen, Imperial College, London, England**

The protein Apocrustacyanin C is a member of the lipocalin family of proteins, which binds to certain pigments that are widely distributed in plants and animals. Knowledge of the structure of the lipocalins will enable scientists to engineer these proteins to produce carriers that will bind more strongly to the pigment crocetin, which has anticancer properties.

Crystal Structure Analysis of the Bacteriophage Lambda Lysozyme

**Principal Investigator:
Dr. Jean-Paul Declercq, Université Catholique de Louvain, Belgium**

The bacteriophage Lambda lysozyme is a small protein of 158 amino acids involved in the dissolution of the cell walls of bacteria. Investigators are seeking information about the method of destruction employed by this organism.

Crystallization of RNA Molecules Under Microgravity Conditions

**Principal Investigator:
Dr. Volker Erdmann, Free University of Berlin, Germany**

Ribonucleic acid (RNA) molecules have diverse biological roles, which include carrying genetic information or amino acids to the ribosomes during protein synthesis and participating as constituents of the ribosomes as they carry out biological functions. Also, RNA molecules may exhibit enzymatic activities. Because of the large mass of its molecules, it has been extremely difficult to crystallize RNA molecules on Earth.

Crystallization of the Protein Grb2 and Triclinic Lysozyme

**Principal Investigator:
Dr. Arnaud Ducruix, CNRS, Universite Paris Sud, France**

Grb2 is an adaptor protein involved in the transfer of signals from one cell to another. Crystallographers have cloned and analyzed Grb2 in ground-based laboratories, but these ground-based crystals do not diffract better than 3.5 angstroms (Å). Better resolution is expected from space-grown crystals. The tetragonal form of lysozyme crystals that was grown in space on earlier missions was not significantly different than those grown on Earth; however, experiments performed on lysozyme crystals grown in the APCF during the Second International Microgravity Laboratory (IML-2) mission in July 1994 indicated that several crystals were essentially perfect single domains, with rocking curve widths as small as 12 arc seconds (about 3×10^{-3} degrees). (The smaller the value of the rocking curve width, the greater the degree of perfection in the crystal.) Investigators want to extend the study to the triclinic form of lysozyme, expecting to reach values as low as 10^{-4} degrees.

Microgravity Crystallization of Thermophilic Aspartyl-tRNA Synthetase and Thaumatin

**Principal Investigator:
Dr. Richard Giegé, CNRS, Strasbourg, France**

Investigators want both to continue and expand the IML-2 crystallization studies on thermophilic aspartyl-tRNA synthetase and to crystallize the plant sweetening protein, thaumatin. While both proteins are biochemically stable and are purified easily, they also have significant structural and behavioral differences; therefore, they make interesting subjects for comparative crystallography studies. In addition, thaumatin tastes extremely sweet when consumed by humans. Since it appears to be non-toxic, non-carcinogenic, and low in calories, it may be a strong substitute for common table sugar.

Crystallization in Space of Octarellins, de novo Designed (alpha/beta)-Barrell Proteins, and of a Mutated Human TIM Forming a Monomeric (alpha/beta)-Barrell Structure

**Principal Investigator:
Dr. Joseph Martial, University of Liège, Belgium**

The long-term goal of investigators is to design a tridimensional "scaffold" onto which binding and/or active sites for selected peptide sequences could be engineered, eventually producing receptor antagonists that could be used as therapeutic agents for the treatment or prevention of disease. Before this can be accomplished, more information is needed concerning the

rules governing protein folding and structure stabilization. Resolution of the three-dimensional structure of crystals of the synthetic protein, octarellin, may provide these data.

Crystallization in a Microgravity Environment of CcdB, a Protein Involved in the Control of Cell Death

Principal Investigator:
Dr. Lode Wyns, Free University of Brussels, Belgium

Clarification of the structure and mode of action of the CcdB protein may lead to the design of new antibiotics and anti-tumoral drugs. Specifically, crystal quality needs to be improved and a systematic twinning problem solved. In addition, researchers want to crystallize two specific serine-to-cysteine mutants (Ser74Cys and Ser94Cys), which have not produced crystals large enough for data collection.

A Multivariate Analysis of X-ray Diffraction Data Obtained From Glutathione S Transferase

Principal Investigator:
Dr. Lennart Sjölin,
University of Göteborg, Sweden

One of the more important components of the debate over the need for a convection-free environment for enhanced crystal quality is the comprehensive analysis of the X-ray data from space-grown crystals and from controls, using a variety of statistical techniques to analyze multiple sets of data. By collecting numerous X-ray data sets from each crystal batch, parameters describing crystal perfection can be studied through a statistical comparison of the crystals grown in space to controls grown on Earth. Various hypotheses can be thereby accepted or rejected with a predetermined statistical significance.

Protein Crystal Growth: Light-driven Charge Translocation Through Bacteriorhodopsin

Principal Investigator:
Dr. Gottfried Wagner,
University of Glessen, Germany

Bacteriorhodopsin converts light energy to voltages in the membrane of photoenergetic microorganisms that are chemically and genetically distinct from bacteria and higher living organisms. Resolution of the three-dimensional structure of this protein will help scientists understand the mechanisms used to convert light energy to energy for growth.

Crystallization of Ribosomes

Principal Investigator:
Dr. Ada Yonath, Max-Planck Laboratory for Ribosomal Structure, Hamburg, Germany

Ribosomes are responsible for the translation of the genetic code to proteins. While they are the only organelles in living cells to have been crystallized, most of the Earth-grown crystals are very thin and crack upon handling, causing severe difficulties in data collection and evaluation. The growth chambers of the APCF are almost tailor made for growing this type of protein crystal and may result in crystals of improved internal order, morphology, size, and mechanical properties. The facility also may allow scientists to control specific properties of the crystal's structure and form.

Crystallization of Sulfolobus Solfataricus Alcohol Dehydrogenase

Principal Investigator:
Dr. Adriana Zagari, University of Naples, Italy

Alcohol dehydrogenase (ADH) is an enzyme that occurs in large amounts in the livers of mammals, where it plays an important role in several physiological functions, including the breakdown of alcohol. Mammalian ADH is unstable at high temperatures or in the presence of organic solvents, properties that limit its biotechnological application to the synthesis of organic compounds. ADH from Sulfolobus solfataricus, a bacterium that thrives at high temperatures, has greater thermal stability, however, and is scarcely affected by the presence of organic solvents. Given these properties, the enzyme is a good candidate for industrial applications.

Crystallization of Turnip Yellow Mosaic Virus, Tomato Aspermy Virus, Satellite Panicum Mosaic Virus, Canavalin, Beef Liver Catalase, Concanavalin B

Principal Investigator:
Dr. Alexander McPherson,
University of California,
Riverside, California

Canavalin, catalase, and concanavalin B are being studied to determine the effects of microgravity on protein crystal growth by evaluation of the size, habit, quality, defects, and diffraction properties, including the resolution limit and mosaic spread of the crystals. Three very large proteins, Satellite Panicum Mosaic Virus (with a diameter of 170Å) along with Turnip Yellow Mosaic Virus and Tomato Aspermy Virus (with diameters of approximately 280Å) are being studied to verify the theory that the impact of altered transport properties in microgravity should be magnified in proportion to the decreased diffusivity of such large molecules.

Crystallization of the Epidermal Growth Factor (EGF) Receptor

Principal Investigator:
Dr. Wolfgang Weber, University of Hamburg, Germany

The receptor for the epidermal growth factor is increasing in its importance as a prognostic factor for a series of human malignancies. Knowledge of the three-dimensional structure of this receptor would open the possibility of tailoring appropriate drugs for the treatment of numerous types of tumors. At the present time, however, the crystal structure of only one hormone receptor (growth hormone) and none of the growth factor receptors have been solved.

The Structure of the Membrane-Embedded Protein Complex Photosystem I

Principal Investigator:
Dr. Wolfram Sänger, Free University of Berlin, Germany

Protein complexes Photosystem I and Photosystem II are responsible for the primary conversion of visible light into chemical energy in water-oxidizing photosynthesis. The objective of this experiment is to elucidate the complete arrangement of chlorophyll molecules, which perform this conversion process in the most efficient way.

Crystallization of Visual Pigment Rhodopsin

Principal Investigator:
Dr. Willem de Grip, University of Nijmegen, Netherlands

Visual pigments like rhodopsin are the primary photoreceptor proteins for a variety of light-regulated processes, such as vision, circadian entrainment, and photoperiodic reproductivity. Analysis of the protein crystals is needed if scientists are to unravel the molecular mechanisms responsible for these processes.