LMM-Biophysics

Objective

Conduct Macromolecular Biophysics (MMB), Protein CrystalGrowth (PCG) experiments with PI specific sample modules operated in the FIR/LMM. Manual mounting of frozen samples.

Relevance/Impact

• A protein crystal is a specific protein repeated over and over ahundred thousand times or more in a perfect lattice.
• These proteins control aspects of human health and understandingthem is an important beginning step in developing and improvingtreatments for diseases.
• The space station provides a unique environment where we canimprove the quality of protein crystals. While we cangrow highresolutioncrystals both in space and on the ground, those grown inspace are often more perfectly formed.

Development Approach

• The LMM is designed for autonomous operation through scripts and ground commanding. Crew time is required for initial installation and check out in the Fluids Integrated Rack (FIR), sample change out, and removal from FIR.
• A ground simulator is available for testing.

LMM Biophysics 1

LMM Biophysics 1 (The Effect of Macromolecular Transport of Microgravity Protein Crystallization)

Experimental Approach of the LMM Biophysics 1 Investigation:

Four aqueous proteins, one membrane protein (P-glycoprotein) and one virus (tobacco mosaic virus, TMV), are selected with the proteins covering a broad range of molecular weights (i.e. ~10kDa to as high as 1,250kDa) and tobacco mosaic virus (TMV) which exhibits a particle diameter of 18.0 nm and a length of ~300 nm. Purified batches of protein and virus particles are produced and isolated in different aggregate populations as outlined in the paragraphs below. Inclusion of P-glycoprotein (an integral membrane protein) and TMV is based on experience within the Center for Biophysical Sciences and Engineering (CBSE) expressing and purifying large quantities, as well as crystallizing this particular membrane protein and virus. Other potential protein candidates include cytochrome c (~12kDa), Glutathione–S-transferase (31kDa) and Thioredoxin (16kDa), α-chymotrypsinogen (25kDa), equine or bovine serum albumin (both ~66kDa), and Xenorhabdus-A, XptA1 (1,250kDa). The CBSE has significant experience expressing, purifying and producing crystals of each of these proteins. The final proteins

selected are primarily based on the stability evaluation of the higher order aggregates of each protein. Dr. DeLucas's laboratory has access to more than 100 different aqueous proteins covering the molecular weight range discussed above. Thus, additional candidates are available if needed.

Specific Aim 1 of LMM Biophysics 1 (aggregate incorporation into growing protein crystals):

As noted in the introduction section, purified proteins generally exist in solution with some small percentage of aggregates (i.e. dimers, trimers, tetramers, etc.). The LMM's confocal fluorescent microscope is used to estimate the percent incorporation of aggregates into growing crystals of several different proteins.

Specific Aim 2 of LMM Biophysics 1 (comparison of µg versus 1-G crystal growth rates):

Specific Aim-2 is accomplished using the identical protein preparation methods as for specific aim-1. Once the growing crystals are identified via the microscope (or any LMM light microscope with 25x to 50x magnification), a series of images is taken over a period of 3 to 5 days to measure the crystal growth rates for each protein candidate. The growth rates for crystals grown in the microgravity environment are compared with 1-G control experiments for the same proteins. The effect of microgravity versus 1g on crystal growth rates is assessed for proteins of different molecular weights, and for protein solutions containing higher order protein aggregates. Solutions containing crystals are maintained in optical cells at either 4°C, or 22°C, and returned to earth for x-ray crystallographic analysis to assess crystal quality.

Specific Aim 3 of LMM Biophysics 1 (compare defect density/quality of crystals grown at different rates in 1g):

Dr. Christian Betzel (co-investigator from Univ. of Hamburg) recently developed a novel technology (Xtal ControllerTM) that provides a unique combination of diagnostic and control capabilities allowing real-time manipulation of the crystallization drop composition. Evaporation rates and crystal growth rates can be adjusted, thereby enabling a detailed comparison of crystal quality versus crystal growth rates. This unique crystallization system is utilized to achieve Specific Aim-3. Analysis of the quality of crystals grown at different rates is performed using atomic force microscopy [37, 38], and x-ray diffraction [11, 21]. The Xtal Controller is located in Dr. Betzel's laboratory at the University of Hamburg. Both Dr. Betzel's and Dr. DeLucas' labs can use this unique system to precisely control key crystallization variables (i.e. protein concentration, temperature, precipitant concentration, additive concentrations) that affect the approach to nucleation, the nucleation event, and the crystal growth phase (figure 2) for each protein studied. Subsequent to the initial set up, which is performed locally, the crystallization experiment can be manipulated and controlled remotely, and followed by both groups on line. As crystal growth rate experiments for each protein are completed using the Xtal Controller, the resulting protein crystals are analyzed via x-ray diffraction and atomic force microscopy (AFM). The x-ray analysis is conducted at the Deutsches Elektronen-Synchrotron (DESY) in Hamburg, Germany using the high brilliant radiation of the storage ring PETRA III, and at the Argonne Synchrotron Facility in Chicago, Illinois. All AFM studies are conducted by Drs. DeLucas and Martyshkin using the facility available in Dr. Martyshkin's lab in the Physics Building at UAB. A portion of crystals produced in Dr. Betzel's laboratory are transported to the DeLucas' laboratory in special thermally insulated containers by Dr. Betzel. In the event that freshly grown crystals must be analyzed prior to one of the planned regular research exchange visits, crystals are to be express mailed to DeLucas' laboratory in special thermally insulated containers (a routinely used method to transport crystals maintained at 4°C or 22°C without any problems).

Science Objectives

Proteins are important biological molecules that can be crystallized to provide better views of their structure, which helps scientists understand how they work. Proteins crystallized in microgravity are often higher quality than those grown on Earth. The Effect of Macromolecular Transport on Microgravity Protein Crystallization (LMM Biophysics 1) studies why this is the case, examining the movement of single protein molecules in microgravity.

Applications

Space Applications

Researchers have crystallized thousands of proteins, but many of them are not high enough quality to allow scientists to view the proteins' three-dimensional structure. One class of proteins, membrane proteins, represent potentially valuable targets for development of new drugs to treat disease, and previous research has suggested that microgravity may improve the quality of this class of important proteins. This investigation improves understanding of the physical processes that enable high-quality crystals to grow in space, where Earth's gravity does not interfere with their formation.

Earth Applications

Crystallizing proteins allows scientists to determine their three-dimensional structure, which enables a better understanding of how proteins work and how they are involved in disease. Protein structure can be used to design new drugs that interact with the protein in specific ways. This investigation provides new insight into how microgravity affects protein crystal growth and quality, benefiting researchers studying protein structure to create new drugs to fight diseases.

Gallery

LMM Biophysics 2

Light Microscopy Module Biophysics-2 (LMMBIO-2)

Research Overview

The main objective of Light Microscopy Module Biophysics-2 (LMMBIO-2) is to understand why protein crystallization experiments in microgravity have often generated unexpectedly low or high numbers of crystals. Both of these outcomes may negatively affect experiments designed to obtain a small number of well-separated crystals for x-ray structure studies.

This unpredictability has been associated with the suppression of buoyancy-driven convection. The conclusion is supported by data indicating the protein crystal nucleation reaches a maximum at an optimal convection velocity. Developing a better understanding of the effects of solution convection on crystal nucleation is a crucial issue in several fields of science and engineering. LMMBIO-2 also tests whether solution convection enhances or suppresses the formation of the dense liquid clusters, within which the crystal nuclei form.

These tests require characterization of the dense liquid clusters at different rates of convection, including its complete absence, and are only possible in microgravity. The Light Microscopy Module (LMM) aboard the International Space Station (ISS) is used to observe the formation of these clusters. Also, a newly-

developed method, called differential dynamic microscopy, is used in order to quantify the dynamics of the dense liquid clusters. Variation in solution composition and the rate of convection are used to identify the cluster formation mechanisms.

Description

The main focus of Light Microscopy Module Biophysics-2 (LMMBIO-2) investigation is to better understand the effects of shear flow on nucleation precursors and therefore, on crystal nucleation. In a microgravity environment, nearly zero-flow states within homogeneous and phase-separating specimens can be maintained for long time periods, providing a baseline against which sheared fluids can be compared. This project is unusual in that the Research Team is also testing a fundamental hypothesis about proteins in solution; that, at moderate to high concentrations, (1) a very small fraction of the protein exists in a dense liquid phase in the form of Brownian particles, each a cluster of roughly 105 protein molecules undergoing liquid-like dynamics, and (2) crystal nucleation occurs when a small, ordered domain forms within the highly concentrated liquid-state environment of one of these particles.

The Research Team presented evidence from shear flow in a droplet that shear affects the precursors in such a way that crystal nucleation occurs most efficiently at a specific strain rate. The hypothesis is that shear leads to ordering by (a) creating local anisotropy within a dense liquid cluster, and (b) by changing the conformation of the protein so that nonpolar residues are exposed, and “hydrophobic” interactions are enhanced. Defining and characterizing dense liquid clusters has significant scientific merit, not only for the understanding and control of crystal nucleation, but also because low volume fraction dense liquid clusters may be universal in concentrated systems.

Science Objectives

The Light Microscopy Module Biophysics-2 (LMMBIO-2) investigation characterizes the behavior of dense liquid clusters at different rates of convection, utilizing differential dynamic microscopy. In microgravity, near-zero flow states can be maintained for long time periods, providing a baseline against which to compare sheared fluids. LMMBIO-2 also tests whether, at moderate to high concentrations, a fraction of a protein exists in a dense liquid phase, and whether crystal nucleation occurs when a small, ordered domain forms within the concentrated liquid-state environment of one of these particles.

Science Results

Find your best microscopic technique to observe tiny and changing physical phenomena aboard the International Space Station (ISS). Researchers have discovered that Differential Dynamic Microscopy (DDM) is a better approach than Dynamic Light Scattering (DLS) for examining small and large particles in dilute mixtures. DDM may be used in the future to measure the rate at which different physical phenomena changes, like the speed of sedimentation and diffusion.

Applications

Space Applications

Defining and characterizing dense liquid clusters is significant for the understanding and control of crystal nucleation. In addition, low volume fraction dense liquid clusters may be universal in concentrated systems. These processes can contribute to development of new materials for use in future spacecraft.

Earth Applications

Light Microscopy Module images provide data that help scientists and engineers understand the forces that control organization and dynamics of matter at microscopic scales. The organization and movement of matter on the microscopic level profoundly affects the macroscopic world and understanding such processes contributes to development of more efficient materials and machines for application on Earth and in space.

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LMM Biophysics 3

LMM Biophysics 3 (Growth Rate Dispersion as a Predictive Indicator for Biological Crystal Samples Where Quality Can be Improved with Microgravity Growth)

Scientists use X-ray crystallography to view molecules that are too small to be seen under a microscope; but this requires crystallizing them, which is difficult to do on Earth. Observing crystallized proteins allows scientists to determine how they are built, which can explain how they work or how other molecules, such as drugs, might interact with them. Growth Rate Dispersion as a Predictive Indicator for Biological Crystal Samples Where Quality Can be Improved with Microgravity Growth (LMM-Biophysics-3) studies ground-based predictions of which crystals benefit from crystallization in microgravity, where Earth's gravity does not interfere with their formation.

LMM Biophysics 4

LMM Biophysics 4 (The Effect of Macromolecular Transport of Microgravity Protein Crystallization)

Proteins are important biological molecules that can be crystallized to provide better views of their structure, which helps scientists understand how they work. Proteins crystallized in microgravity are often higher in quality than those grown on Earth. The Effect of Macromolecular Transport on Microgravity Protein Crystallization (LMM Biophysics 4) studies why this is the case, examining the movement of single protein molecules in microgravity.

LMM Biophysics 5

LMM Biophysics 5 (Solution Convection and the Nucleation Precursors in Protein Crystallization)

Solution Convection and the Nucleation Precursors in Protein Crystallization (LMM Biophysics 5) tests whether solution convection – movement of molecules through the fluid – enhances or suppresses formation of the dense liquid clusters from which crystals form. The investigation uses images from the Light Microscopy Module (LMM) to characterize these clusters at different rates of convection in order to identify cluster formation mechanisms. This helps determine why protein crystallization investigations in microgravity often generate unexpectedly low or high numbers of crystals.

LMM Biophysics 6

LMM Biophysics 6 (Growth Rate Dispersion as a Predictive Indicator for Biological Crystal Samples Where Quality Can be Improved with Microgravity Growth)

Scientists use X-ray crystallography to view molecules that are too small to be seen under a microscope; but this requires crystallizing them, which is difficult to do on Earth. Observing crystallized proteins allows scientists to determine how they are built, which can explain how they work or how other molecules, such as drugs, might interact with them. Growth Rate Dispersion as a Predictive Indicator for Biological Crystal Samples Where Quality Can Be Improved with Microgravity Growth (LMM Biophysics 6) studies ground-based predictions of which crystals benefit from crystallization in microgravity, where Earth's gravity does not interfere with their formation. In this experiment, 2 proteins of interest in cancer treatment and radiation protection are to be studied.