Increment 57/58 Science Symposium
Light Microscopy Module Biophysics - 4 (LMM-B4)

Presented by:
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LMM-B4

NASA Research Announcement (NRA) - 2013
Macromolecular Biophysics

The Effect of Macromolecular Transport on Microgravity Protein Crystallization

- Experiment to be performed in the FIR LMM.
- Compare incorporation of protein aggregates into growing protein crystals on ISS and on earth
- Measure growth rates in 1g versus microgravity (μg) for different size aggregates of proteins.
- Compare the defect density and crystal quality via fluorescent-based atomic force microscopy and X-ray diffraction quality of crystals grown at different rates in a 1g environment.
- Launch scheduled for June 2018 on SpaceX-15 (B4)

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[AeroSpace Corp.]  
PI / Provide flight samples, science requirements, and data analysis

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Science Background and Hypothesis
Investigation goals and objectives
Measurement approach
Importance and reason for ISS
Expected results and how they will advance the field
Earth benefits/spin-off applications
Science Background and Hypothesis

Science Background

There are over 100,000 proteins in the human body and an estimated 10 billion throughout the global environment. To fully understand how they work and how they interact with each other, it is necessary to determine their 3-dimensional structure. This is most often done through analysis of X-ray diffraction of quality crystals.

A newer method, using analysis by neutron diffraction, determines the position of hydrogens within a protein structure and enables more accurate determination of biochemical reactions taking place within and between proteins.

- Neutron diffraction requires very large, quality crystals, greater than 1 millimeter$^3$ in volume.
- Fewer than 100 unique neutron structures of proteins have been reported in the Protein Data Bank, as compared to over 90,000 X-ray diffraction structures.

Quality data for both X-ray diffraction and neutron diffraction structure determination requires crystals of high quality with few defects, and this is often the bottle-neck for crystallographers.

It is particularly difficult to grow high quality crystals of membrane proteins which have the desired qualities. It is estimated that 20 – 30% of all genes in all genomes are integral membrane proteins and that membrane proteins are the targets of over 50% of all modern medicinal drugs.
Review Publication: 63 Space Shuttle flights by more than 150 investigators from the international scientific community (Kundrot, C.E., et. al., “Microgravity and Macromolecular Crystallography”, (2001), *Crystal Growth and Design*, vol 1, No. 1, p. 87-99):

- 179 proteins flown prior to STS-95, 36 proteins (20%) obtained the highest diffraction resolution to date from the microgravity-grown crystals.

- Direct correlation between success rates and the number of times a protein is flown
  - Of 81 proteins that flew only once, only two showed increased diffraction quality. In contrast, more than 50% of the proteins that had four flight opportunities produced crystals with increased diffraction quality.

- The magnitude of the diffraction improvement varied significantly but in more than 50% of the cases, the microgravity-grown crystals resulted in at least a doubling of the amount of diffraction data.
Science Background and Hypothesis

“The crystals grown under microgravity were up to 20 times larger than all crystals grown on earth previously. The largest microgravity-grown crystal was 4mm long and 1.5 mm in diameter. The native data collected from a microgravity-grown crystal formed the basis for the improved crystal structure of PSI at 4 angstroms resolution”

Large single crystal of photosystem I, grown in the space shuttle Columbia during the USML-2 Mission
Science Background and Hypothesis

How Does Microgravity Influence Protein Crystallization?

• Elimination of sedimentation and convection (this can enhance protein crystallization).

• During the nucleation event, the solution in the immediate vicinity of the growing crystal becomes depleted of protein molecules as the crystal nucleates and begins to grow.
  • Local supersaturation at the surface of the growing crystal is decreased, favoring crystal growth over additional nucleation (resulting in bigger and fewer crystals).

• Growth of a crystal through the addition of a new protein molecule to the surface is dictated by diffusion rather than buoyancy-induced convective sources.
  • Protein molecules traverse the droplet at a very slow rate providing more time for crystal lattice alignment (producing a higher quality crystal).
Science Background and Hypothesis

• Protein crystals have been grown in microgravity for over 3 decades. Numerous research publications and review articles have documented beneficial effects of a microgravity environment for protein crystallization.

• However, the exact mechanism for this beneficial influence remains unclear. This project will investigate two theories:

**Hypothesis**

Improved quality of microgravity-grown protein crystals is the result of two macromolecular characteristics that exist in a buoyancy-free, diffusion-dominated solution:

1. Slower crystal growth rates, due to slower protein transport to the growing crystal surface

2. Predilection of growing crystals to incorporate protein monomers versus higher protein aggregates due to differences in transport rates
LMM-B4 Investigation Goals and Objectives

**Aim-1:** To compare the incorporation of different size protein aggregates (monomers vs. dimers, trimers, etc.) into growing crystals for proteins with a range of molecular weights (20 kDa to 1200 kDa or more) for μg and 1 g environments.
- Detection of aggregates in the crystal will be accomplished using LMM’s confocal fluorescence microscope
- Percentage incorporation of larger aggregates will be used to assess the effect of molecular filtering based on differences in diffusion rates

**Aim-2:** Measure crystal growth rates in 1 g vs. μg for proteins with a range of molecular weights (< 20 kDa to > 1200 kDa)
- Protein crystallization will be imaged and photographed using LMM for growth rate analysis

**Aim-3:** Compare the crystal diffraction quality and defect density for crystals grown at different (controlled) rates in a 1 g environment.
- Control of nucleation and subsequent growth rates by the Xtal Controller crystallization system (made available through collaboration with Dr. Christian Betzel, University of Hamberg)
- Analysis by x-ray diffraction studies and atomic force microscopy
We will be using a flight-hardened Commercial-Off-The-Shelf (COTS) microscope
[pictured on next page]
and a
Macromolecular Biophysics sample module
[pictured later]
Measurement approach

Light Microscopy Module (LMM) in the Fluid Integrated Rack (FIR)

LMM in the Closed Position or Operating Configuration

LMM in the Open Position or Installation/Service Configuration
Payload specific and multi-user hardware customizes the FIR in a unique laboratory configuration to perform research effectively.

Payload Specific Hardware
- Sample Cell with universal Sample Tray
- Specific Diagnostics
- Specific Imaging
- Fluid Containment

Multi-Use Payload Apparatus
- Test Specific Module
- Infrastructure that uniquely meets the needs of PI experiments
- Unique Diagnostics
- Specialized Imaging
- Fluid Containment

Light Microscopy Module

FCF Fluids Integrated Rack
- Power Supply
- Avionics/Control
- Common Illumination
- PI Integration Optics Bench
- Imaging and Frame Capture
- Diagnostics
- Environmental Control
- Data Processing/Storage
- Light Containment
- Active Rack Isolation System (ARIS)

Philosophy: Maximize the scientific results by utilizing the existing LMM capabilities. Develop small sample modules and image them within the LMM
Light Microscopy Module (LMM)  

LMM-B4 Sample Assembly Prototype that will contain up to 8 square 100mm capillaries
Experimental Procedure for Capillary Observation and Photo Documentation on ISS

Pre launch 30 days
- Load protein and solutions in capillaries at UAH
- Snap freeze capillaries and store -80°C at UAB

Upon Arrival to ISS, Integrate launch transfer to -80°C freezer on ISS or transfer capillaries to existing ISS 80°C freezer

30 mins prior to thaw, observations begins
- Remove from -80°C storage on ISS and place capillary cartridge in LMM to thaw solutions at ambient ISS temperatures. This automatically activates the crystallization process.
- Position Capillary #1 under bright field or fluorescent microscopy for observation at 10X magnification.
- Repeat photo documentation every 2-6 hours (depending on protein). Locate and save location of 10-20 crystals as they appear, for subsequent photos. Periodic downlink to science team.

Repeat process for 7-14 days
- Increase mag 20X to 50X, photo documentation of 10-20 crystals in 2-12 hour intervals
- Downlink photo documentation
- After imaging is completed, remove capillary cassette and store at 22°C ± 2.0°C (ISS ambient temperature is acceptable)
- Maintain at 22 ± 2.0°C throughout re-entry/retrieval and transport to PI
More than 125 research publications and review articles provide compelling evidence of the beneficial effect of a microgravity environment for protein crystallization. However, many scientists remain unconvinced that microgravity has a positive impact, primarily due to limitations in previous experiments:

• Delays in shuttle launch resulted in degradation of some proteins prior to flight
• Short $\mu$g duration in shuttle flights either led to no crystallization (nucleation is dramatically slower in $\mu$g), or crystals that were too small to support diffraction studies (crystal growth is slower in $\mu$g)
• Inconsistencies in protein batch purification between $\mu$g samples and ground-based samples
• Experiments lacked proper control experiments or results were not statistically convincing
• Incomplete understanding of the mechanisms for how microgravity is beneficial to protein crystallization

This proposal will investigate the influence of two theories proposed by the scientific community to explain the beneficial influence of microgravity on protein crystallization.
Expected results and how they will advance the field

Long-duration protein crystal growth experiments on the ISS with photo documentation, and subsequent analysis of the comparisons between 1g and µg crystals, will enable a more complete understanding of why proteins and other macromolecules often form more perfect crystals in microgravity than they do on earth.
Earth benefits / spin-off applications

In spite of major advances in the rate of crystallizing novel proteins there are more than 9,000 previously crystallized human aqueous and membrane proteins, targeted by NIH as extremely important for structure determination, for which the crystals are of insufficient quality to yield a structural solution (based on NIH Structural Genomics websites).

Structural biology of protein-protein complexes and integral membrane proteins are currently a high NIH priority due to their importance for systems biology, disease mechanisms and structure-guided drug development.
LMM-B4

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BACKUP SLIDES
## Mission Success Criteria for LMM-B4 (DeLucas)

<table>
<thead>
<tr>
<th>Success Level</th>
<th>Accomplishment</th>
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| Minimum Success     | (1) For one of the 6 different protein/virus samples: must be able to compare the approximate percentage incorporation of higher macromolecular aggregates in crystals grown in microgravity versus 1g (this specific aim utilizes LMM’s planned confocal fluorescent microscopy capability)  
                      (2) At the completion of both flights, for one of the 6 different protein/virus samples: must be able to accurately compare crystal growth rates in microgravity versus 1g (this specific aim utilizes LMM’s bright field microscopy capability) |
| Significant Success | (1) For 4 of the 6 different protein/virus samples: must be able to compare the approximate percentage incorporation of higher macromolecular aggregates in crystals grown in microgravity versus 1g (this specific aim utilizes LMM’s planned confocal fluorescent microscopy capability)  
                      (2) At the completion of both flights, for 4 of the 6 different protein/virus samples: must be able to accurately compare crystal growth rates in microgravity versus 1g (this specific aim utilizes LMM’s bright field microscopy capability) |
| Complete Success    | (1) For all of the 6 different protein/virus samples: must be able to compare the approximate percentage incorporation of higher macromolecular aggregates in crystals grown in microgravity versus 1g (this specific aim utilizes LMM’s planned confocal fluorescent microscopy capability)  
                      (2) At the completion of both flights, for all of the 6 different protein/virus samples: must be able to accurately compare crystal growth rates in microgravity versus 1g (this specific aim utilizes LMM’s bright field microscopy capability) |