Macromolecular Crystallization in Microfluidics for the International Space Station

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ABSTRACT

At NASA's Marshall Space Flight Center, the Iterative Biological Crystallization (IBC) project has begun development on scientific hardware for macromolecular crystallization on the International Space Station (ISS). Currently ISS crystallization research is limited to solution recipes that were prepared on the ground prior to launch. The proposed hardware will conduct solution mixing and dispensing on board the ISS, be fully automated, and have imaging functions via remote commanding from the ground. Utilizing microfluidic technology, IBC will allow for on orbit iterations. The microfluidics LabChip® devices that have been developed, along with Caliper Technologies, will greatly benefit researchers by allowing for precise fluid handling of nano/pico liter sized volumes. IBC will maximize the amount of science return by utilizing the microfluidic approach and be a valuable tool to structural biologists investigating medically relevant projects.

Keywords: Macromolecular, Crystallization, Microfluidics, Protein, NASA

1 INTRODUCTION

280 miles above our heads, there exists a near perfect vacuum wherein temperatures can range between ± 200 °F, and a view that can only be described by poets. If you travel at a speed of 5.3 miles/second you will orbit the Earth once every 92 minutes and experience the weightless environment of μg. In this harsh, yet unique environment, NASA has chosen to assemble the International Space Station (ISS). This facility allows for long term experimentation to be carried out in an environment similar to an Earth bound laboratory with one difference, reduced gravity.

The study of proteins and other macromolecular molecules has been a growing interest in understanding diseases, aging, cellular repair, metabolism regulation, and genetic malformations. Understanding these molecules can give rise to new medications and treatments to correct or eliminate conditions that can effect the human body. One of the primary methods to study macromolecular molecules is to crystallize the molecule and place the crystal into an x-ray beam. The resulting diffraction pattern can tell scientists how the molecule is structured, identify bonding sites for potential chemicals to form medications, determine how that molecule can interact with other molecules, and to understand why this crystal formed in order to gain knowledge to help crystallize other molecules. But, the x-ray diffraction information is only as good as the crystal (size & internal order of the crystal). Two major issues that may affect the crystal growth process are convective flow and sedimentation, both influenced by gravity. Scientists have theorized that growing crystals in a μg environment would yield larger and more perfect crystals, thus allowing them to extract even better information.

2 FLIGHT HISTORY

Attempts have been made in the past, on many shuttle missions, to test the μg crystal growth theory. But several inherent problems have limited the investigation process. First, crystallization may take three to four weeks in orbit. The average shuttle mission has been approximately 12 days with the longest flight lasting 17 days. The second issue has been the available resources on the shuttle. Some of these are power, heat removal, and crew time. Most flight experiment hardware (see Figure 1), require that protein and precipitant solutions are premixed, but stored separate from each other.

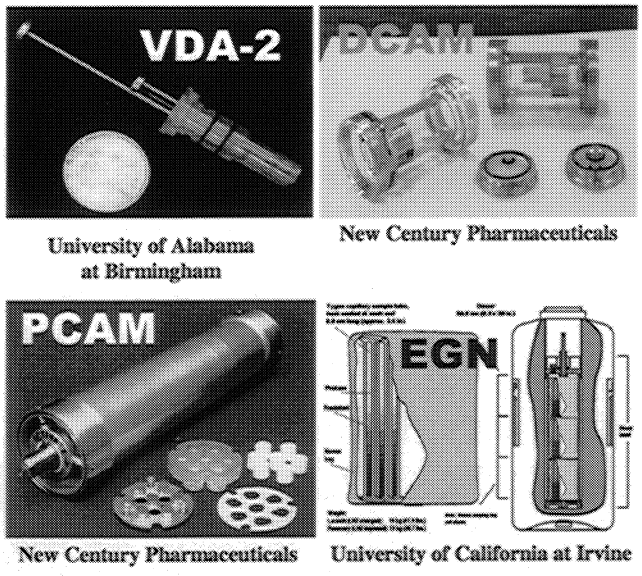

Figure 1: Current Protein Crystallization Flight Experiment Configurations [1]

Once on orbit these experiments are activated by the crew, by the turn of a knob or flip of a stitch, thus activating the equilibrium process. At a predetermined time the astronaut reverses the earlier action and the experiment is de-activated. The experimenter on the ground must then wait for the return of the experiment to assess the results. There have been a few attempts, with more complex, to gather information during growth on orbit, see Figure 2.

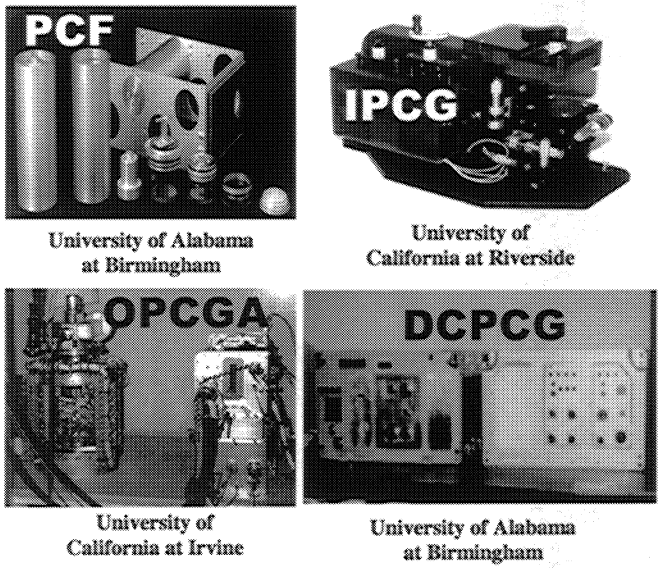

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Figure 2: Advanced Protein Crystallization Flight Experiment Configurations [1]

But the configurations require more resources and still require the solutions to be premixed on the ground. And, if an experimenter wishes to make changes to the mixtures or conditions of the experiment, they will have to wait for another flight (ranging from 6 months to 2 years). Despite the lack of definitive data as to why these macromolecules grow better in μg, data indicates that roughly 20% do. Additionally, the chances for success in μg increase significantly with multiple flight opportunities see Figure 3.

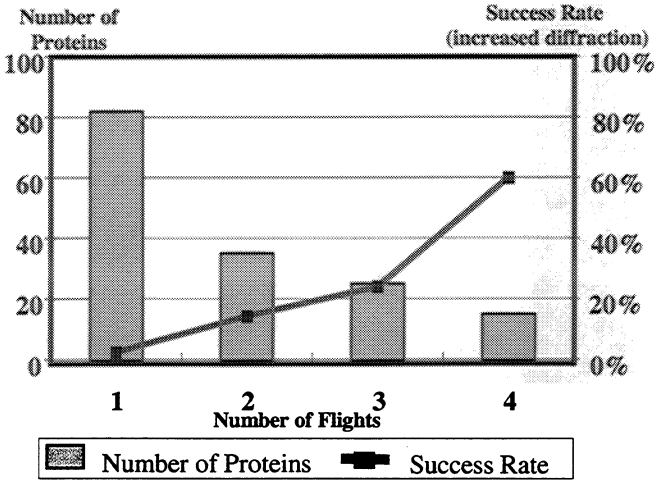


Figure 3: Number of Proteins Flown with Crystal Improvement by Number of Times Flown [2]

Unfortunately, most researchers do not want to wait around for years to have the opportunity to re-fly their experiment. For most researchers the time required to do "iterative experiments" in space is not in line with the pace of ground-based research.

3 NRC REPORT

In 2000 the National Research Council (NRC) was asked to review the data collected, to date, concerning protein crystallization in space. Key among their findings [3] was that there is not a clear answer as to whether growing crystals in a μg environment exhibited marked improvement or not. The response was partially due to all of the different crystal growth devices/methods used, and the lack of comparable data from those devices. Based on the NRC's recommendations, and the apparent need for on orbit iterations, the Iterative Biological Crystallization (IBC) project was initiated at NASA's Marshall Space Flight Center.

4 IBC REQUIREMENTS

To ensure that the IBC project was capturing the diverse needs of the prospective user community, an informal local science team and a more formal Science Advisory Group (SAG) was assembled. The SAG, made up of members from academics, industry, and government, meets biannually to review the science requirements and IBC design concepts. It is intended that their input is extensive, and representative of the broad-spectrum of investigators that will utilize IBC.

Requirements Overview

Top level science requirements have been developed as summarized:

- Provide an automated capability for investigators to mix from up to eight solutions.
- Provide a growth cell that accommodates vapor diffusion, batch, and liquid-liquid growth techniques.
- Provide remote imaging of growth cells.
- Accommodate at least ten investigators simultaneously, each having a minimum of 150 experiments (about 1500 total crystal growth experiments) per increment.
- Provide resources for at least three iterations per increment (i.e. the time between crew change-out).
- Ensure for thermal controlled and quiescent incubation of growth cells.
- Monitor the acceleration/vibration environment in the incubation area.
- Ensure sample integrity (no cross contamination).
- Provide safe sample return to Earth and/or hand-over for crystal harvesting, cryo-preservation, and further x-ray diffraction study.

To date, IBC has held three SAG reviews. Information from the reviews has refined IBC's requirements and helped clarify/confirm the development path.

5 IBC PAYLOAD & OPERATIONS

From the onset, the IBC project has paid particular attention to payload operations (see Figure 4). Included are pre-flight thru post-flight activities, all monitored via the interactive IBC web site.

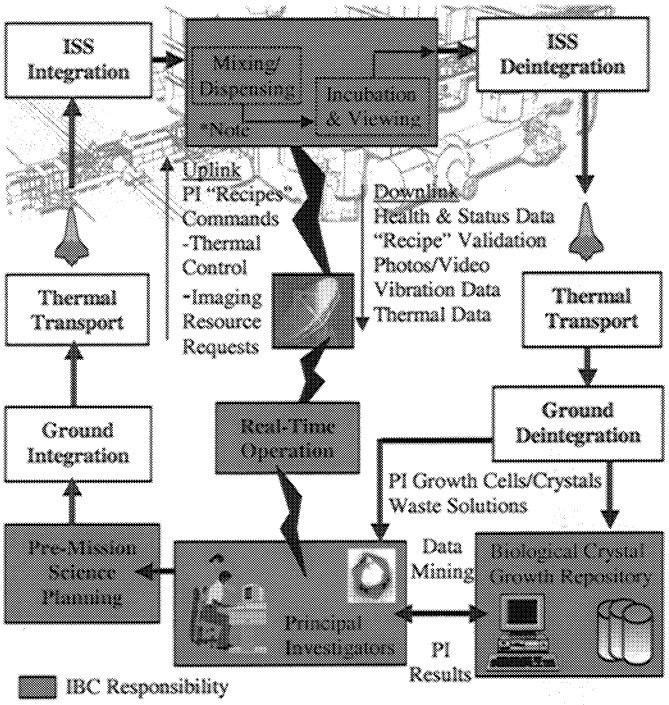


Figure 4: IBC Payload Operations Cycle Overview [4].

Currently the IBC flight payload is to be housed in half of an EXPRESS Rack (see Figure 5).

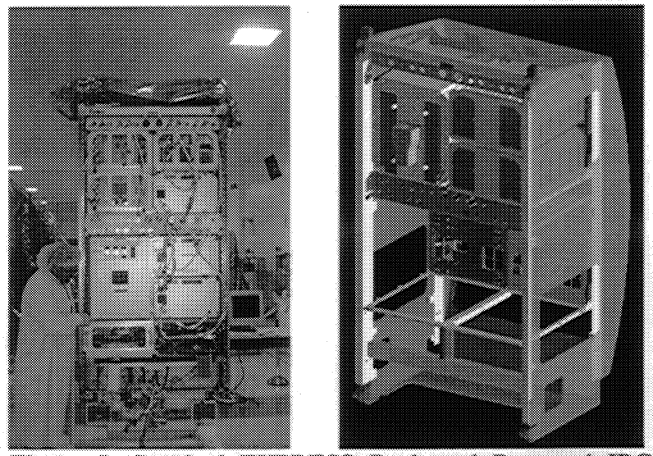
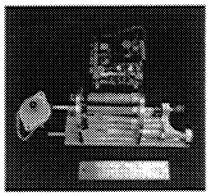


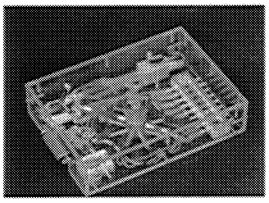
Figure 5: Standard EXPRESS Rack and Proposed IBC Payload Configuration

The IBC payload configuration consists of two single lockers and one double locker. A single locker is shown as one of two units in the upper right hand corner of the right hand picture of Figure 5. The double locker is shown just to the left of the two single lockers in the same picture. A double locker is roughly (19 x 17 x 20) inches. The two single lockers will house the computer and support electronics for IBC. This leaves the double locker to house the equipment to perform the experiments and the 1500 experiments. In order to meet the science requirements and live with the physical limitations, a new way of performing macromolecular crystallization is needed. The greatest challenge is the handling of the experiment fluids (i.e. dispensing, mixing, isolating, etc.). Early attempts were made using different forms of off the shelf equipment (see Figure 6).

Syringe Pump Configuration



Modified Syringe Pump Configuration



Miniaturized Pump Configuration (size of video cassette)

Figure 6: Early Fluidic Handling Breadboards

Further research lead to the newly immerging micro-fluidic technology. Microfluidics holds the potential to perform all of the sciences requirements within the limited confines of the payload carrier. However, the efficacy of the microfluidic approach had to be proven and the minimum volume to yield a usable crystal determined. After surveying the microfluidic market, Caliper Technologies in Mountain View California was the clear choice for partnering in the IBC development. A joint feasibility study was performed utilizing an off-the-shelf chip developed by Caliper. A protein solution was placed in one well of a "T" channel configured chip and a precipitating solution in another well. These two solutions were then sent through the microfluidic channels, mixed, and deposited in the remaining well. The results were a positive indicator that the approach worked. It was possible to harvest a crystal and do x-ray diffraction, see Figure 7. In short, it was possible to utilize microfluidics to grow crystals in reduced volume droplets; moreover, crystals grown using microfluidics were of equal quality (x-ray data

of 1.8 Angstroms) to those grown by conventional laboratory methods.

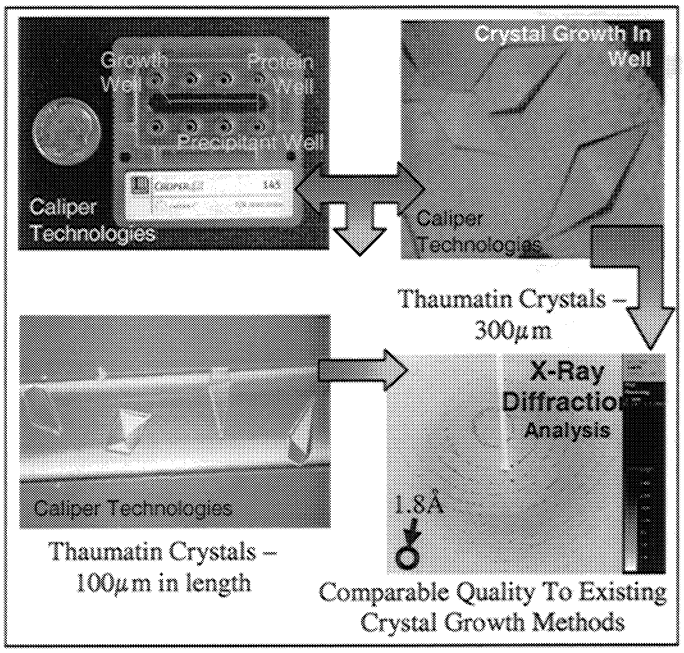


Figure 7: Feasibility Study

Based on these successful findings, designs were started on developing a chip specifically for the batch method. After 6 months of joint development the NS374 chip (see Figure 8) was completed and is being evaluated. The two main goals in testing this chip are to study the microfluidic effects of mixing different viscosity fluids and expand upon the number of systems using this technique.

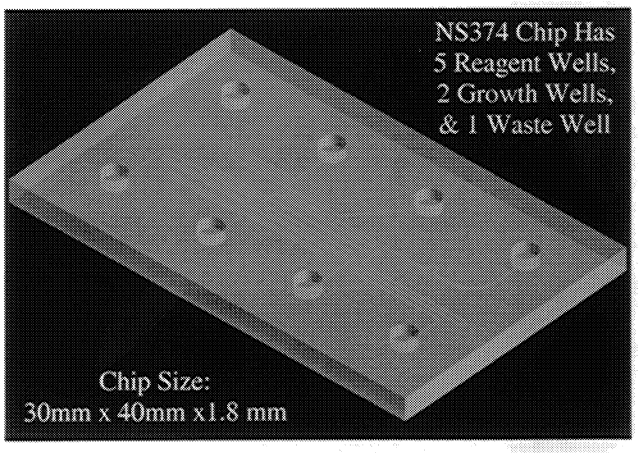


Figure 8: Protein Crystallization Development Chip

The NS374 chip has served as a proof of concept/development chip, but a new chip design will be needed to meet all of the science requirements. Current efforts are considering such features as; micro valves for fluid isolation prior to mixing and isolation of samples during the incubation process, in channel micro pressure sensors to determine viscosity changes in various solution mixtures (to better control fluid flow), integrated optic features to aid in viewing the crystals, micro pumps for the control/movement of the fluids on the chip, and sealing methods that will allow better interfaces to the macro world. Concept ideas that have been considered are shown in Figure 9.

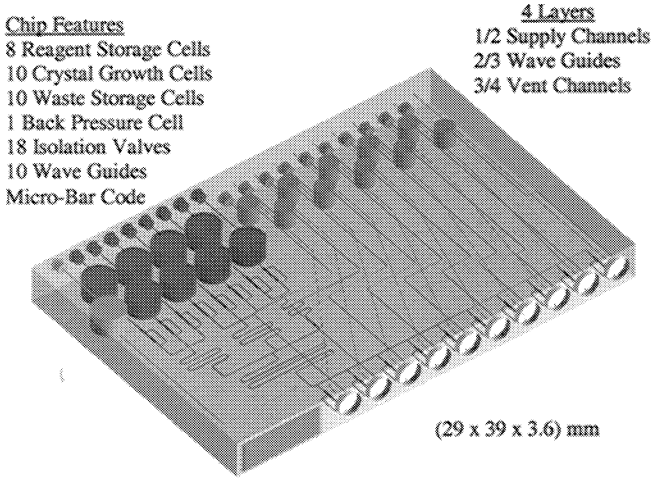


Figure 9: IBC Chip Concepts

This type of chip will allow the requirements to be met, but there is much work to be done.

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