Commercial ITA Biomedical eXperiments (CIBX-2): ITA Commercial Urokinase Cancer Research Project

STS-107 Final Report *August 19, 2004*

Principal Investigators:	**John M. Cassanto**	Instrumentation Technology Associates, Inc. (ITA) 108 Ashland Drive, Exton, PA 19335
	Dr. Allen Edmundson	Oklahoma Medical Research Foundation Oklahoma City, OK
	Dr. John Bognacki	Nu-Millennium Research, Inc. New York
	Dr. Dennis Morrison	NASA Johnson Space Center Houston, Texas
	Ulises R. Alvarado	ITA Consultant, Exton, PA

I. Introduction

A. Abstract

The Commercial ITA Biomedical eXperiments (CIBX-2) payload was the latest in a series of cutting edge microgravity experiments for cancer research conducted by Instrumentation Technology Associates, Inc. (ITA) during the last ten years. The payload flew as part of a Space Act Flight Agreement between NASA and ITA. The payload was unique in that it was the only microgravity payload on the STS-107 mission that was a fully commercial venture. The CIBX–2 payload encompassed a total of eleven separate experiments. The prime commercial experiment was an attempt to grow large, stable, well-ordered protein crystals of the enzyme urokinase to enable X-ray diffraction studies to determine its three-dimensional molecular structure of the active site of the enzyme. Once the structure is known, a molecule can be designed to block the active site, preventing metastatic tumor cells from migrating to other locations in the body. Secondary experiments included: the Bence-Jones protein crystallization experiment for bone cancer research, commercial experiments (e.g., microencapsulation drug delivery for cancerous tumors), and student experiments from ten schools as part of ITA and Space Outreach's Hands-On Student Microgravity Experiments Program.

B. Highlights

- Urokinase protein of 99% purity was flown on STS-107 to grow higher purity protein crystals than on previous missions.
- Urokinase is an important protein to understand for development of pharmaceuticals to stop the spread of Cancer.

C. Hypothesis

Large, well-structured urokinase protein crystals can be grown in the near-zero convection microgravity environment by using ultra-pure solutions of the protein. The high quality of the crystals will enable the three-dimensional characterization of the entire urokinase molecule through the use of X-ray diffraction.

ITA's CIBX-2 payload on the STS-107 Columbia mission was a re-flight of the CIBX-1 STS-95 mission, utilizing identical hardware and experiment protocol but using ultra-pure urokinase. It is known that urokinase protein crystals were grown on the CIBX-1 STS-95 mission, but the diffraction patterns obtained from the Brookhaven synchrotron were poor. Scientific analysis revealed that the urokinase purity used on STS-95 was approximately 70% and it was determined that a repeat of the flight protocol and hardware with ultra-pure urokinase would be the key to producing a protein crystal that would diffract. Accordingly, urokinase of 99% purity was used on the CIBX-2 payload flown on the STS-107 Columbia Shuttle mission.

D. Investigation Objective

The investigation objective was to grow large, stable, well-ordered protein crystals of urokinase so that X-ray diffraction studies could be performed to determine its three-dimensional molecular structure. The molecular structure of urokinase has been only partially identified. When the entire structure is defined, it will facilitate the development of drugs to inhibit cancer metastasis.

E. Background/History of Project

This was the eighth Shuttle mission in an ongoing ITA commercial space research project to produce high-quality crystals of the enzyme urokinase to continue our cancer research program in the microgravity environment of space. It constituted the prime commercial experiment to be performed on Columbia under the auspices of the current NASA-ITA Space Act Flight Agreement.

ITA's urokinase protein crystal research experiment has flown on previous Shuttle missions dating back to STS-43. It has taken many Shuttle flights to optimize the sample conditions and to develop the proper experiment protocol to be able to grow urokinase crystals sufficiently large and stable to not go back into solution prior to successful x-ray diffraction studies. Figure 1 shows a top-level chronology of some of the urokinase flight results prior to the Columbia mission. The CIBX-2 payload on the STS-107 Columbia mission was to be the culmination of the program and was to be essentially a repeat of the CIBX-1 mission which did in fact grow a large urokinase protein crystal which did not diffract.

II. Methods/Research Operations

A. Method/Protocol

Urokinase crystals were grown on STS-95 on ITA's CIBX-1 payload, however the crystals did not produce a useable diffraction pattern. Our science team, after detailed analysis, concluded that the crystals did not diffract due to the low purity (70%) of the urokinase utilized on the STS-95 mission. An extraordinary effort was made to repeat the space experiment using identical hardware and protocols and other variations on the CIBX-2 STS-107 mission with the highest purity urokinase available. Accordingly, we utilized 99% purity urokinase and followed the identical protocol on this mission.

B. Functional Objectives

Urokinase is an enzyme secreted by tumor cells to enable them to migrate through tissues from the primary tumor to a remote site where they begin replicating to form another metastatic tumor. The urokinase experiments were designed to obtain large, perfect crystals to enable ground-based x-ray diffraction studies to determine the 3-D structure of the active site of the enzyme. The functional objectives of CBIX-2 were to produce several urokinase crystals of X-ray diffraction quality.

C. Hardware Items Used; Descriptions of PI-provided Hardware

The experiments utilized two of the commercial hardware devices designed and built by ITA, Inc., within the ITA built CIBX-2 payload:

- Hardware 1: Capillary tubes within the Liquids Mixing Apparatus (LMA)

- Hardware 2: Sample cavity wells in ITA's Dual Materials Dispersion Apparatus (DMDA). Urokinase protein crystal growth experiments for this mission utilized the "double diffusion" technique.

Of the two hardware devices, only the DMDA's were found in the debris (Figures 2 & 3). The DMDA consists of an aerospace aluminum housing. Contained within the housing are two blocks of inert materials with fluid wells machined into facing sides of the blocks. A single well on one block has two corresponding wells on the opposite block. The blocks are offset so each well on one block faces the inert wall of the other block. In space, the flight crew activates the DMDA and the blocks are repositioned to align the wells with each other. A diagram showing the DMDA cavity wells are shown in Figure 4. A detailed description of ITA's hardware can be found online at: http://www.itaspace.com/hware.html.

Both of these hardware units and experiment protocol techniques have been flown previously and have been utilized to grow very small urokinase protein crystals or larger crystals that went back into solution before X-ray diffraction analysis could be implemented within days of harvest. A total of 33 urokinase experiment samples were flown on the CIBX-2 payload: 13 sample wells in the DMDA hardware, and 20 samples in the LMA hardware (Table 1).

III. Results

A. List of Pre-, In- and Postflight Anomalies

No anomalies occurred.

B. Completeness/Quality of Data

Within 24-hours of the Columbia break-up, ITA engineers from the CIBX launch team identified their hardware shown in TV news programs (Figure 2) and a newspaper photograph [Ref. 2]. The two DMDA hardware units miraculously survived re-entry but showed evidence of elevated temperatures and charring (Figure 3). However, the exterior aircraft aluminum housings and seals that isolated the sample wells held up well during re-entry, impact, and throughout the months in the KSC hangar before the data recovery efforts commenced (Figure 5). As of May 6, 2003, samples from the DMDA experiment in the CIBX-2 payload were recovered, but none of the samples contained urokinase crystals upon recovery (Figure 6). Manually activated Liquid Mixing Apparatus (LMA) devices were also flown containing urokinase samples as a part of the payload; however, none of these devices have been recovered from the Columbia debris. The quality of the data from all of the eleven primary and secondary experiments flown in the DMDA hardware ranged from fair to good for about half of the CIBX-2 experiments, including growth of microcapsules, inorganic crystals, and biofilms. However, all of the science obtained was degraded somewhat between 50-75% with the exception of urokinase, which completely degraded due to the long time delay (3 months +) between identification of the hardware and the actual recovery of the samples, coupled with the lack of a controlled temperature environment after Shuttle break-up.

C. Tables, Graphs, Figures Index

Table 1. Urokinase Protein Crystal Growth Experiments

Urokinase (uPA)	Experiment Type	Hardware Unit
13 experiments	Type 3 - Double diffusion	DMDA (recovered)
20 experiments	Capillary Diffusion	LMAs (not recovered)

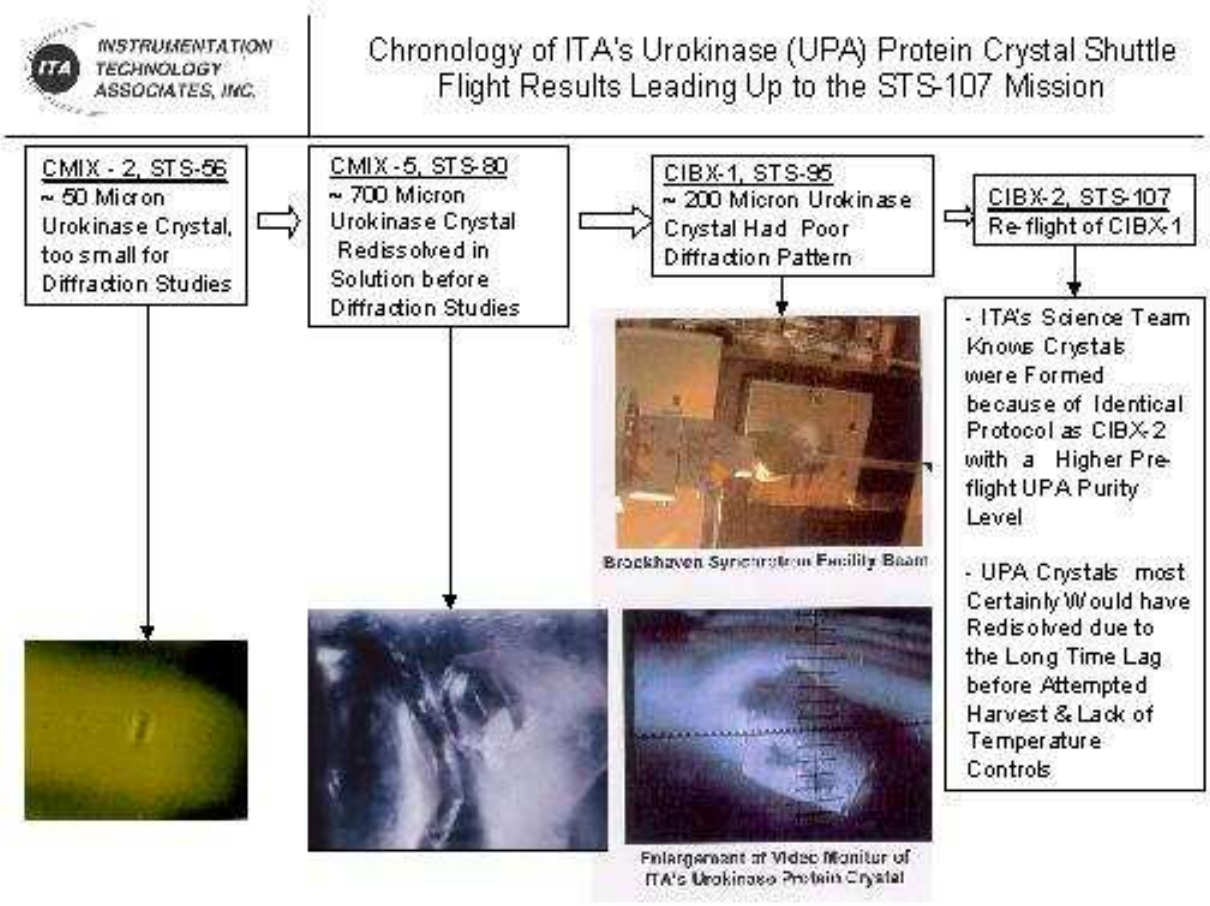

Figure 1. Chronology of ITA's Urokinase Protein Crystal Shuttle Flight Results Leading up to the STS-107 mission.

INSTRUMENTATION TECHNOLOGY ASSOCIATES, INC.

ITA Personnel Identified DMDA Hardware Being Protected by the National Guard within 24 Hours of Columbia's Breakup

Figure 2. Composite picture of recovered hardware in Nacagdoches, Texas. February 2003.

Figure 3. DMDA post-recovery shows charring from re-entry. Photo taken at NASA KSC O&C lab, May 5, 2003.

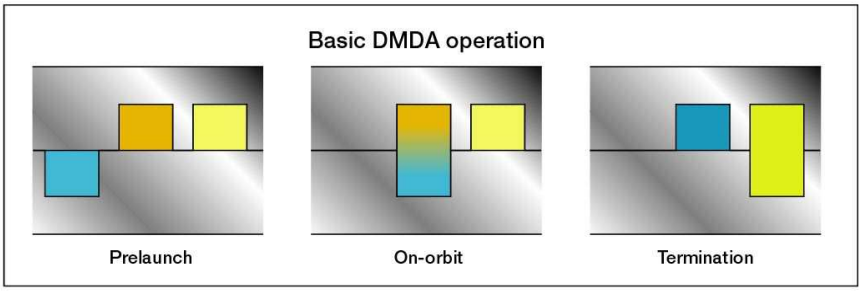

Figure 4. Diagram depicting a cross-section of a set of DMDA experiment specimen Wells and sliding block principle of operation.

Figure 5. ITA's John M. Cassanto and George D'Heilly inspect the recovered DMDA at the NASA KSC O&C building May 6, 2003.

Figure 6. Valerie Cassanto of ITA, Inc., unloads the urokinase Cancer research experiments from the DMDA blocks found during the search for Columbia debris. NASA Photo KSC-03PD-1456, NASA Kennedy Space Center, Florida, May 6, 2003.

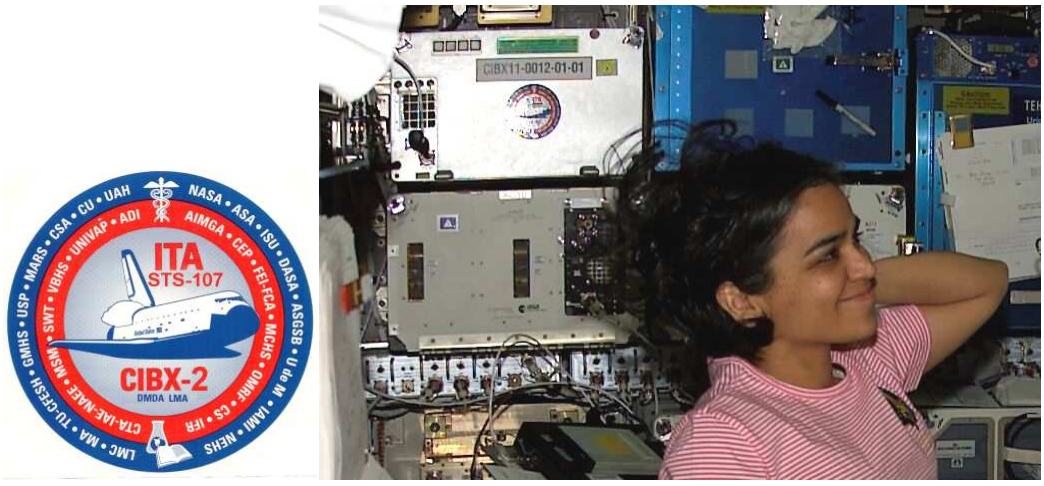

Figure 7. STS-107 astronaut Kalpana Chawla (K.C.) is pictured in front of the CIBX-2 payload (upper left) on-orbit. Photo: NASA.

D. Status of Data Analysis:

All data analysis is complete.

E. Final Investigation Results

On the STS-107 CIBX-2 mission, ultra-pure urokinase (99%) was utilized for the first time in our attempt to grow higher quality protein crystals than those produced on the CIBX-1 STS-95 mission when 70% pure urokinase resulted in lower purity crystals. Our science team believes that urokinase crystals did in fact grow on the Columbia mission based upon the previous CIBX-1 STS-95 results. However, the team unanimously believes that the long time lag (3 months+) before approval was given to harvest the samples and the lack of a controlled temperature environment most certainly caused the urokinase protein crystals to go back into solution.

F. Conclusions/Recommendations

A Herculean effort was made by the ITA team to obtain and process the high value ultra pure urokinase, and to prep the hardware to essentially re-fly the CIBX-1 STS-95 experiment on Columbia. The CIBX-2 payload on STS-107 was to be the shuttle flight to provide the solution to the elusive urokinase structure, which is the key to combating cancer metastasis. The crew of Columbia diligently worked to conduct the various phases of our urokinase cancer experiment on the mission. However, all this effort was dashed when Columbia broke up. Accordingly, the ITA team is ready again to re-fly the urokinase cancer experiment with the identical protocol that we know will produce large well-ordered protein crystals so that the structure can be determined. Accordingly, we are requesting that NASA manifest us on the first available Shuttle that will fly after the "stand down" and retrofit period and/or to fly our cancer experiment on an ISS mission as soon as possible.

G. Earth Benefits/Spin-off Applications

The urokinase experiments were designed to obtain large, perfect crystals to enable ground-based x-ray diffraction studies to determine the 3-D structure of the active site of the enzyme. Once the structure is known, a moleculae can be designed to block the active site, which would prevent metastatic tumor cells from migrating to other locations in the body, or enable targeting those metastatic cells directly with anti-tumor agents or radioisotopes. This approach could lead to the first cell-specific therapy to target metastatic cancer cells rather than the bulk of the non-metastatic primary tumor cells.

IV. Bibliography

[1] "STS-107 Mission After the Mission: Recovery of Data from the Debris of Columbia," A. P. Over, J.M. Cassanto, V.A. Cassanto, L. J. DeLucas, P. Reichert, S. M. Motil, D. W. Reed, F. T. Ahmay, AIAA 2004-285, 42nd AIAA Aerospace Sciences Meeting**,** Reno, NV, 5-8 January 2004.

[2] "First the Air Shook With Sound, And Then Debris Rained Down," New York Times, February 2, 2003, page 1 and page 27.

[3] Instrumentation Technology Associates, Inc. (ITA)
www.ITAspace.com

[4] STS-95 Experiment: Commercial ITA Biomedical Experiments CIBX Press Kit, STS-95
http://www.shuttlepresskit.com/STS-95/experiment33.htm