

# **Biological Macromolecular Crystal Growth**

**NASA ISS Research Academy and Pre-Application Meeting  
August 3-5, 2010**

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## Introduction

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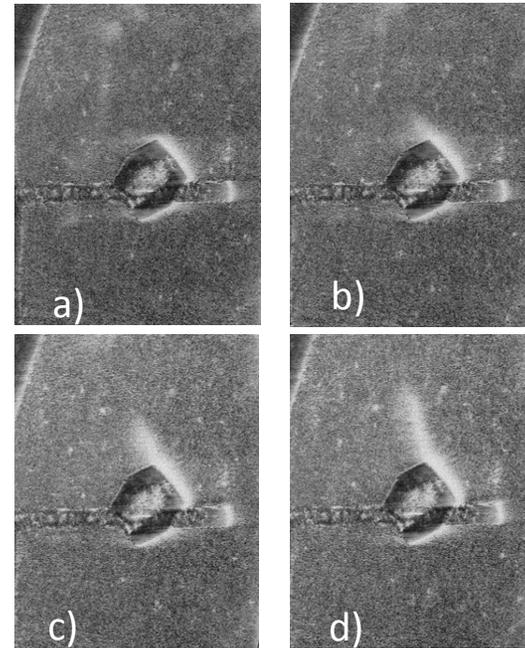
- Biological Macromolecular Crystal Growth (a.k.a., Protein Crystal Growth) research focuses on determining the three dimensional structure of biomolecules
  - Viruses
  - Proteins
  - DNA
  - RNA
- Structural determination provides basis for understanding function and can aid in:
  - Structure-guided design of pharmaceuticals, herbicides, insecticides
  - Further our understanding of life's most fundamental processes (basic biological research)
- Advances in diagnostic equipment and techniques have enhanced determination of new structures, but crystal production of sufficient quality for structural analysis continues to prove challenging to the scientific community



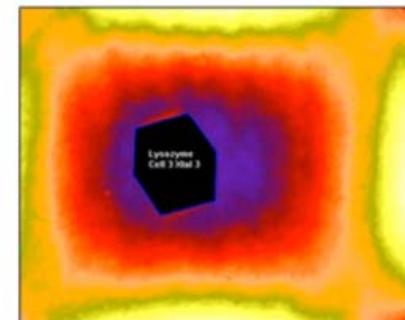
## Why is Microgravity an Ideal Environment?

- As crystals grow, solution around them becomes depleted of sample. On the ground this rises causing turbulent convection.
- Buoyancy driven convection and sedimentation are greatly reduced in the ISS microgravity environment resulting in:

- 1. Formation of stable depletion zone around the growing crystal:** slow consistent addition of molecules to the crystal lattice
- 2. Reduction in secondary nucleation:** fewer crystals competing for solute resulting in larger crystals
- 3. Suspension of growing crystals:** do not settle to bottom of growth chamber (more uniform shape)



Schlieren photograph of a growth plume rising from a lysozyme crystal (pH 4.0, 0.1M sodium acetate, 5% NaCl at 15°C.M.L. Pusey, J. Cryst. Growth, 122, 1-7, 1992) .



Depletion zone around lysozyme crystal (ground based)  
Courtesy: Dr. A. McPherson, UCI



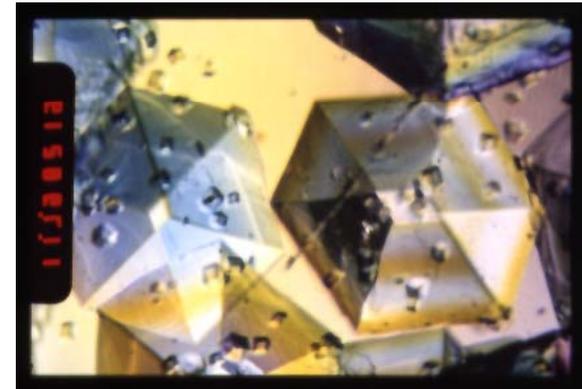
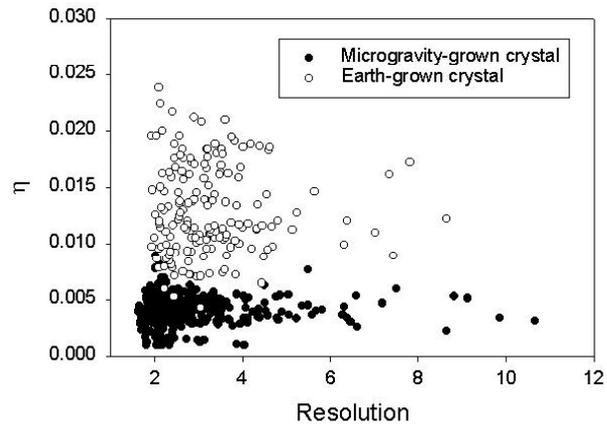
## *Why Crystal Growth in Microgravity?*

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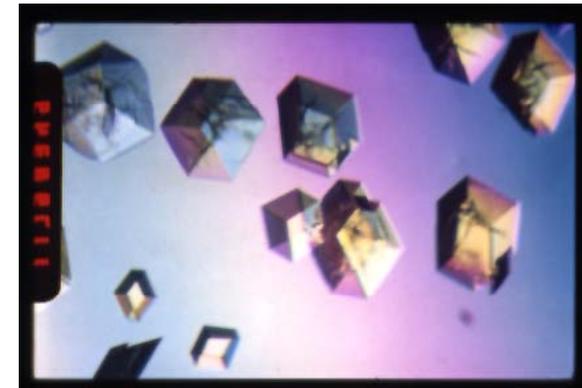
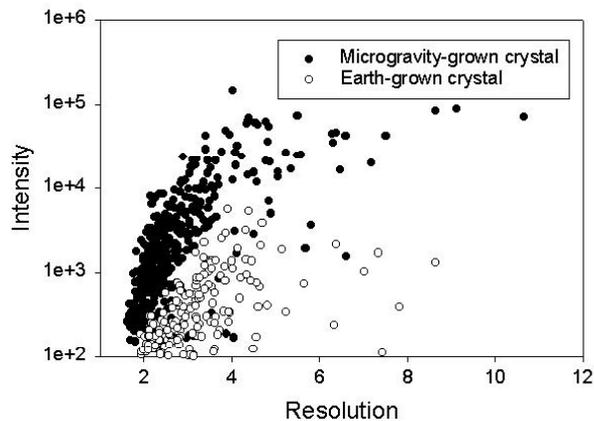
- Microgravity provides an environment which can significantly improve the yield of crystals suitable for diffraction analysis
- Diffraction analysis of crystals grown in microgravity have shown: [Judge et al., Acta Cryst. D61, 763-771 (2005)]
  - Increased resolution
  - Decreased mosaicity
  - Increased crystal volume



# Comparison of Microgravity vs. Ground Grown Insulin Crystals



Microgravity



Ground

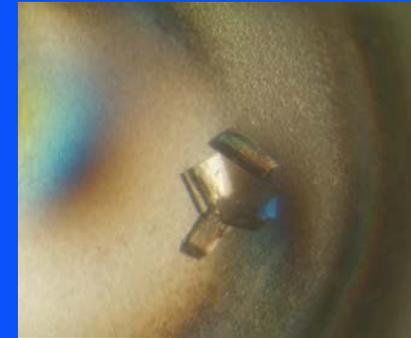
Plots of crystal mosaicity and signal-to-noise for microgravity and ground grown insulin. Shown beside the plots are photographs of the respective crystals. The microgravity crystals are the large crystals above the smaller ground-grown samples. Both images are to the same magnification with the microgravity crystals being approx 2 mm across (Borstahl et al., 2001).



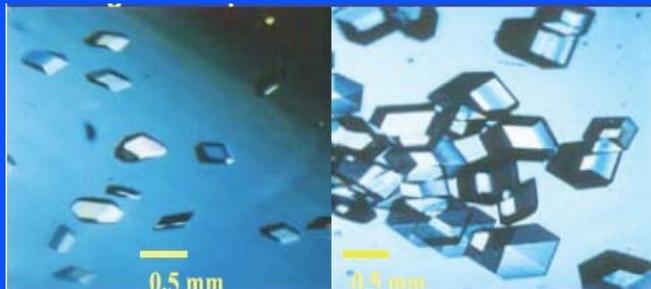
## Examples of Microgravity Grown Crystals

Previous flight investigations have shown that microgravity environment (such as on ISS) is ideal for biological crystal growth

**Antithrombin III** – Dr. Mark Wardell, Washington University of St. Louis This molecule's physiological function is to control blood coagulation in human plasma and is an important target for understanding thrombolytic disease and stroke. **Structure of human antithrombin III was refined at high resolution (1.8 Å) and represents the highest resolution serpine structure determined at that time.** Journal of Molecular Biology (1997) 266, 601-609.



**Microgravity Grown Human Antithrombin III Crystals**



**Human Insulin Crystals**  
Target for Improved Treatment of Diabetes

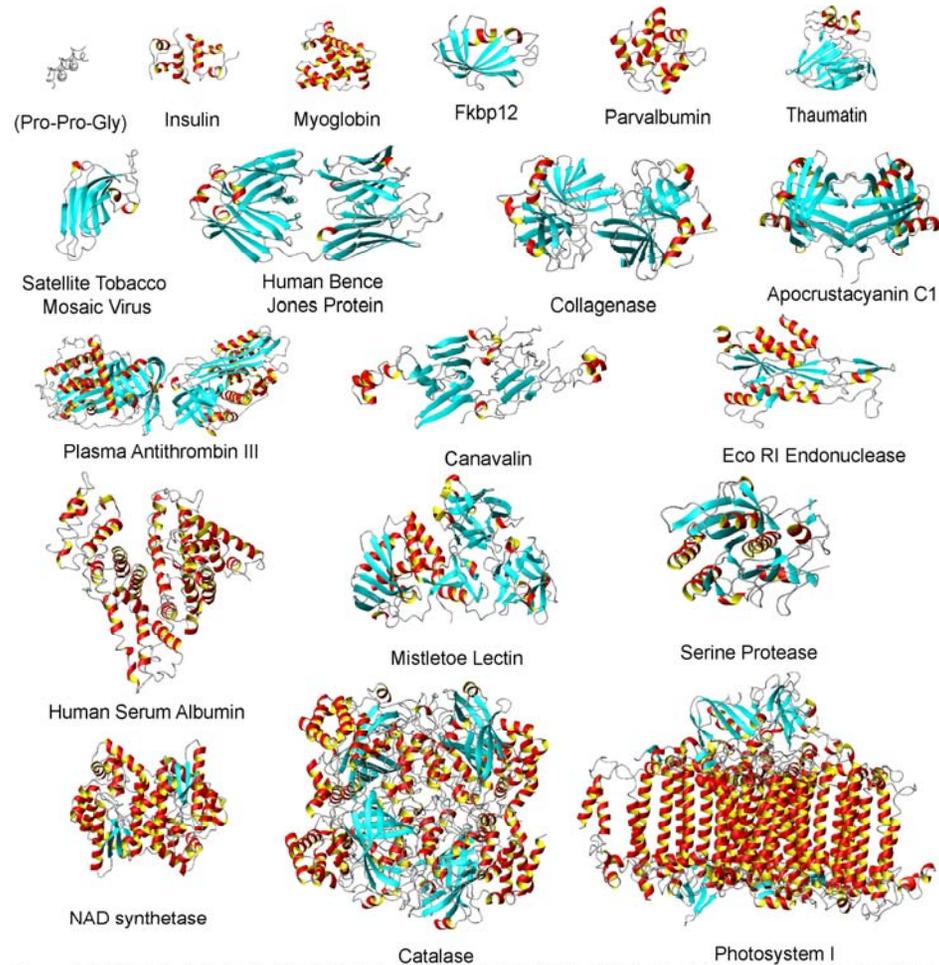
Insulin-drug complex crystals grown on earth and in microgravity. The earth-grown crystals produced an inaccurate map of the protein-drug structure whereas the microgravity-grown crystals revealed an accurate protein-drug structure.  
(Courtesy: Dr. L. DeLucas, UAB CBSE)

In terms of improved diffraction, 5% of samples are improved in the first flight. The more iterations of a sample, the higher the success rate – 15% for two flights, 25% for three and 60% for four (Kundrot et al. 2001, Crystal Growth and Design, 1, 87-99)



# Examples of Microgravity Grown Crystals

## New and Improved Published Macromolecular Structures Resulting from Microgravity Research



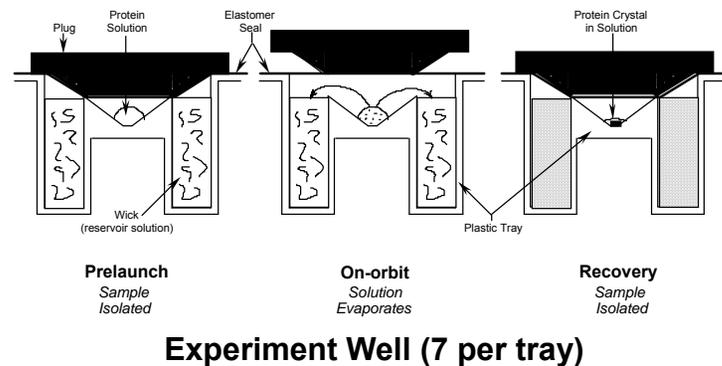
**References.** (Pro-Pro-Gly) - Beriso et al., *Protein Science* (2002), 11, 262-270; Insulin - Smith et al., *Acta Cryst D*, (2003) 59, 474-482; Myoglobin - Miele et al., *Acta Cryst.* (2003) D59, 662-668; Fkbp12 - Wilson et al., *Acta Cryst D*, (1996) 51, 511-521; Parvalbumin - Deleercoq et al., *Protein Science* (1999) 8, 2194-2204; Thaumatin - Sauter et al., *Proteins: Struct. Funct. Genet.* (2002) 46, 146-150; Satellite Tobacco Mosaic Virus - Lanson et al., *J. Molecular Biology* (1996), 271, 37-59; Human Bence Jones Protein - Terczyan et al., *J. Mol. Recog.* (2003) 16, 83-90; Apocrustacyanin - Habash et al., *Acta Cryst D*, (2003), 7, 1117-1123; Plasma Antithrombin III, Skinner et al., *J. Molecular Biology* (1997), 266, 201-; Canavalin - Ko et al., *Acta Cryst D*, (2000), 46, 411-420; Collagenase - Brouin-L'Hermitte et al., *Acta Cryst D*, (2000) 56, 376-378; Eco RI Endonuclease - Rosenberg et al., structure deposited in PDB, publication in preparation; Human serum albumin - Carter et al., *Science* (1990) 249, 302-303; Mistletoe lectin - Krauspenhaar et al., *Acta Cryst D*, (2002) 58, 1704-1707; Serine Protease - Betzel, C. et al., *Biochemistry* (2001) 40, 3080-3088; Catalase - Ko et al., *Acta Cryst D*, (1999) 55, 1383-94; NAD Synthetase - Symensky et al., *Acta Cryst D*, (2002), 58, 1138-1146; Photosystem I - Klukas et al., *J. Biol. Chem.* (1999), 274, 7351-7360. Not included are the Nucleosome Core Particle - Harp et al., *Acta Cryst D*, (2000) 56, 1513-1534; Human interferon-gamma, Ealick et al., *Science* (1991), 252, 698-702; Bacteriophage lysoczyme - Evrard et al., *J. Mol. Biol.* (1998), 276, 151-164. This poster is not inclusive, it does not include many structures of commercial interest where the coordinates and publication have not been released. Images are not to scale. For reproduction of the poster please contact the author.

Edward H. Snell, 2003.



# Investigation Hardware: Protein Crystallization Apparatus for Microgravity

- PCAM utilizes “sitting-drop” vapor diffusion method of crystal growth
- Each PCAM contains 63 individual experiments
- Can accommodate several investigations with differing scientific objectives:
  - Production of crystals for x-ray and neutron diffraction structure determination
  - Better understanding of physical processes in microgravity
- Flight history, has flown on Shuttle and ISS missions : STS-62, -63, -67, -73, -83,- 94, -85, -95, -101, -100, -108, -111, -112
- Can be flown in thermal carrier or in passive stowage depending on target investigations
- Each PCAM activated/deactivated using ratchet wrench from IFM toolkit



PCAMs Installed in Thermal Carrier



Activation/  
Deactivation  
Plate

Experiment  
Tray  
(9 per cylinder)

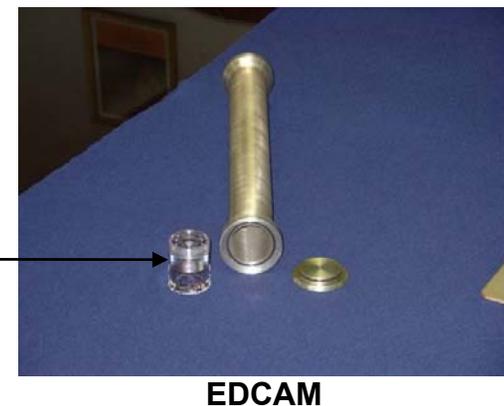
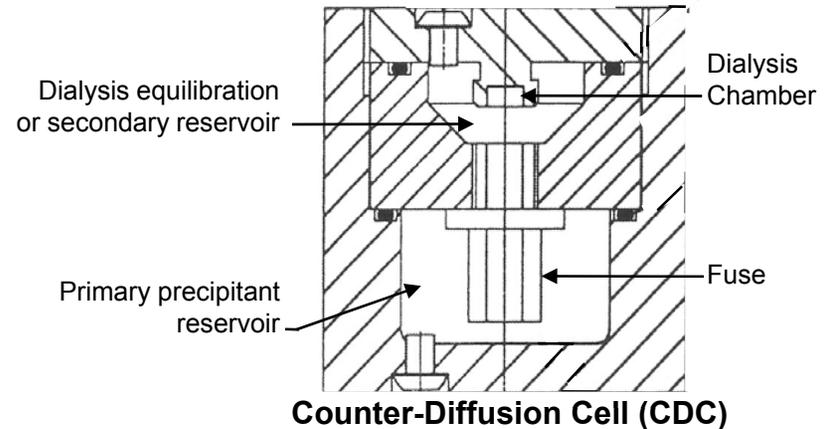
PCAM

Hardware Provider: MSFC & Dr. D. Carter, New Century Pharmaceuticals



## Investigation Hardware: Enhanced Diffusion-controlled Crystallization Apparatus for Microgravity

- Developed to take advantage of long duration experiment opportunities available on ISS
- Counter-diffusion cells can be individually programmed to control rate of approach to supersaturation over periods from several days to months
- Crystals grown by liquid-liquid diffusion or dialysis methods
- Particularly suited for growth of large multi-millimeter crystals required for neutron diffraction
- Growth is diffusion-limited, which in many cases can result in crystals of greater size and perfection for application in structural molecular biology
- Can be flown in thermal carrier or in passive stowage depending on target investigations
- Self-activating, no crew interaction



**EDCAMs Installed in Thermal Carrier**

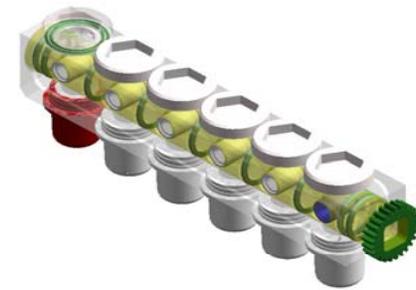
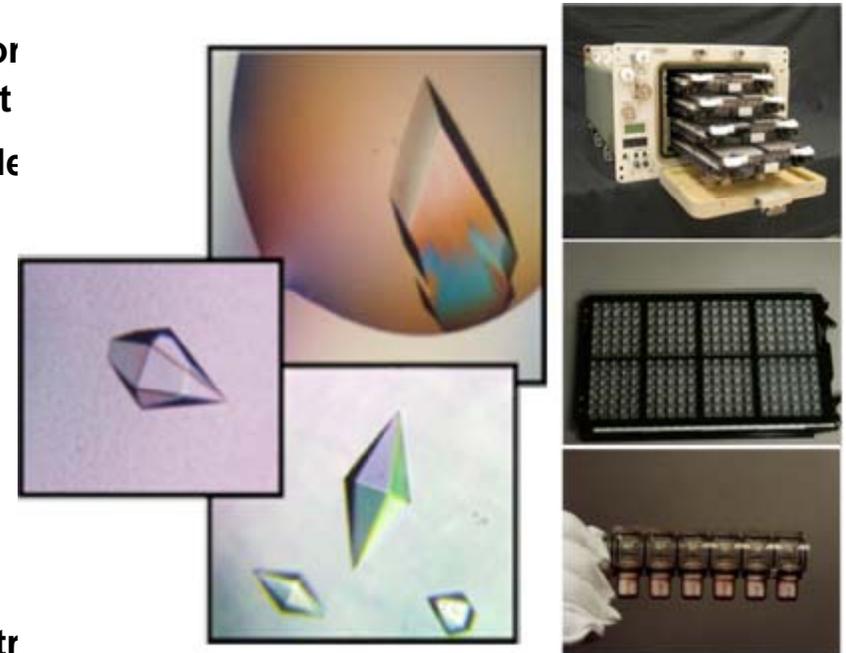
**Hardware Provider: Dr. D. Carter, New Century Pharmaceuticals**



## Investigation Hardware: High Density Protein Crystal Growth

### Features:

- Design allows for multiple PI teams in 1 system
- Shuttle Middeck and ISS-EXPRESS Certified
- Individual Growth Cells available to PI teams for Ground based testing and condition refinement
- 4 Tray configuration provides up to 1008 sample
  - 4 Tray Assemblies per MERLIN.
  - 42 Growth Cells per Tray
  - 6 Sample per Growth Cells
- Enhanced Design of Previous Crystal Growth Experiments.
- Better Thermal Conduction to the Experiment Chambers
- Simple Crew Activation and Deactivation
- Housed inside MERLIN for active Thermal Contr °C to +48 °C Air cooling – 20 Deg C with MTL
- > 65 lbs Total Mass
- 160W Peak, 80W Nominal Power Draw
- Can also be used to support other biology, biotechnology, chemistry, etc. experiments



*Hardware Provider: Dr. L. DeLucas*

*University of Alabama at Birmingham,  
Center for Biophysical Sciences & Engineering*



## Investigation Hardware: Observable Protein Crystal Growth Apparatus

- Measure crystal size & gross growth rates
  - 2-D maps of refractive index utilizing phase shift Mach Zehnder interferometer
  - Record video images view orthogonal to interferometer utilizing video microscopy system
- ExPRESS investigation requiring 4.5 MLEs:
  - Experiment Module (EM)
  - Video Data Acquisition and Control System (VDACS)
  - Video Tape Recorder (VTR)
  - Thermal Enclosure System (TES); *GFE*
  - Ancillary stowage items
- 96 liquid-liquid diffusion growth cells
- Two Polarization Microscopes (PMs) record time-lapsed video of crystal growth
- Two phase shift Mach Zehnder interferometers produce phase maps for post-flight analysis



Experiment Growth Cells



Experiment Module (EM)



Experiment Module (EM) Installed in TES  
(front hatch removed)



## Conclusion

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Access to the International Space Station with mission durations of six weeks to three months will increase the number of protein applications that can be studied and significantly improve the yield of crystals suitable for diffraction analysis, thus providing a greater impact to a broad spectrum of academic and commercial programs.

Excerpt from Nobel laureate Dr. Herbert A. Hauptman (1985 Nobel Prize in Chemistry) via letter to the honorable Dave Minge, U.S. House of Representatives, 23 June, 1995:

*“I was initially quite skeptical about the possible benefits that might come from these efforts but I have clearly seen the advantages that this unique environment has to offer in the area of protein crystal growth”*



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## *Additional Examples of Microgravity Grown Crystals*



# Examples of Microgravity Grown Crystals

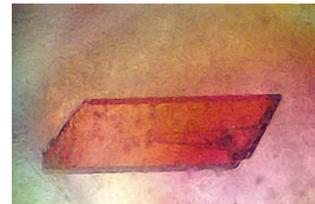
## Gloria Borgstahl, Eppley Institute for Cancer Research, Principal Investigator

NRA: "Searching for the Best Crystals: Integration of Synchrotron-Based Crystal Quality Measurements and Structure Determination"

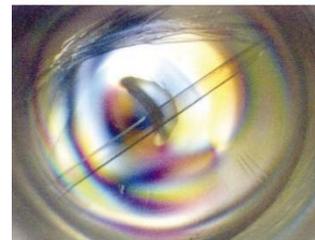
Crystallization target: Fe Superoxide Dismutase  
Superoxide dismutases (SODs) are important antioxidant enzymes that protect all living cells against the toxic superoxide radical. SOD protects the body from the oxidative damage that is associated with aging. When oxidative damage is not prevented, we age and start to have age-related diseases such as heart disease, diabetes and cancer..

## Dr. Daniel Carter, New Century Pharmaceuticals, Inc., Principal Investigator

A major focus of NCP involves detailed studies of the protein human serum albumin, the major protein of the circulatory system. In addition to contributing to colloidal osmotic blood pressure (80%), albumin is involved in the binding and transportation of an incredible variety of small molecule throughout the circulatory system, including most of the pharmaceuticals currently known to mankind. Precise atomic information relating to the ligand interaction with albumin can serve to guide scientists in improving the safety and efficacy of important pharmaceuticals. Highest resolution and quality native data to date by any method.

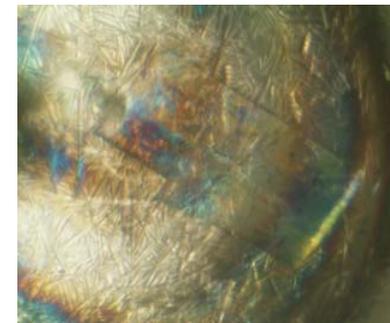
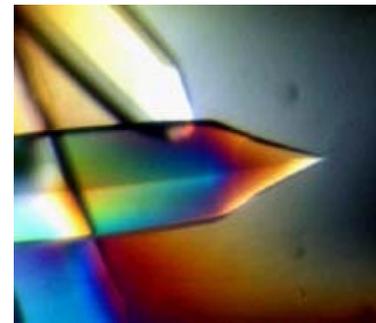


(a)



(b)

Examples of microgravity-grown MnSOD crystals in the PCAM crystallization chamber. (a) Crystal with dimensions 0.45 0.45 1.45 mm. The pink color is due to oxidized manganese in the active site. (b) An example of crystals limited in size to 3 mm in length by the drop volume.



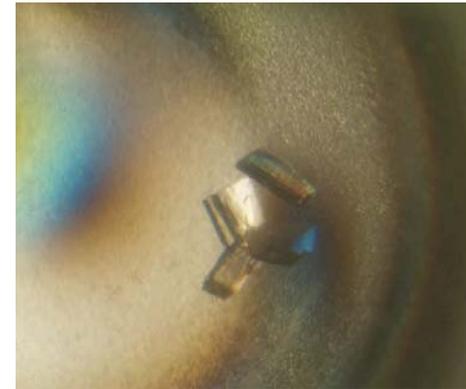
## Examples of Microgravity Grown Serum Albumin Crystals



## Examples of Microgravity Grown Crystals

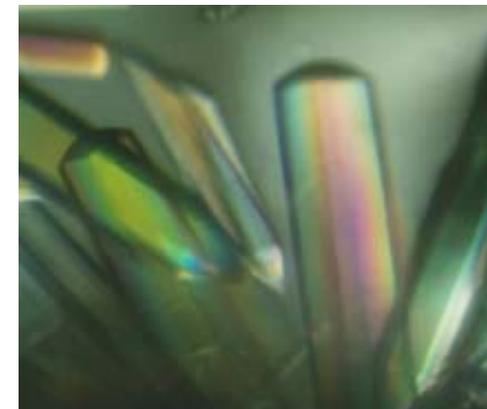
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**Antithrombin III – Dr. Mark Wardell, Washington University of St. Louis** This molecule's physiological function is to control blood coagulation in human plasma and is an important target for understanding thrombolytic disease and stroke. **Structure of human antithrombin III was refined at high resolution (1.8 Å) and represents the highest resolution serpine structure determined at that time.** Journal of Molecular Biology (1997) 266, 601-609.



**Microgravity Grown Human Antithrombin III Crystals**

**Pike Parvalbumin - Professor Jean-Paul Declercq, University of Louvain, Belgium-** Ultra-high resolution crystals of this typical EF-hand protein were obtained from space-grown crystals. These crystals diffracted to 0.91Å. Parvalbumins are proteins found in the muscles, endocrine glands, skin cells, and some neurons of vertebrates. Researchers are exploring the parvalbumin biochemistry and specifically its Ca binding properties related to muscle relaxation. Protein Science 8, (1999)

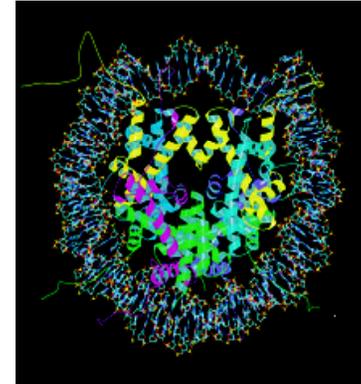


**Microgravity Grown Pike Parvalbumin Crystals**



## Examples of Microgravity Grown Crystals

**Nucleosome Core Particle - Dr. Gerard Bunick ORNL , Principal Investigator** Determined the structure of the nucleosome core particle (NCP) to 2.5 Å resolution using crystals grown in space. NCP is found in all cells containing nuclei. It forms a scaffold around which the DNA is entwined and is important in the process of transcription. Acta Cryst. (2000) D56, 1513-1534.



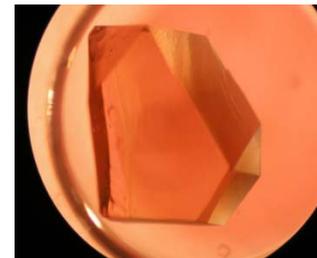
**Nucleosome Core Particle (NCP) Structure**



**Nucleosome Core Particle Flight Crystal**

**D-xylose ketol-isomerase (glucose Isomerase)- Dr. Gerard Bunick ORNL , Principal Investigator**

Glucose Isomerase is an enzyme which is widely used in the food processing industry. The crystals of this enzyme will be a model system for the Spallation Neutron Source at Oak Ridge National Laboratory.



**Xylose Isomerase Flight Crystal**



## Examples of Microgravity Grown Crystals

**Bacteriorhodopsin: Professor Gotfried Wagner, Justus Liebig University, Principal Investigator** Bacteriorhodopsin is a membrane associated photochromic protein of interest in bio-electronics for information storage purposes. "Some exceptionally large individual needles were grown in the DCAM on Mir," Fewer defects, Improved optical perfection, Larger crystal volumes (Individual crystals/crystal clusters 1.9mm/8mm) Microgravity Sci. & Tec. XIII/3 (2002)

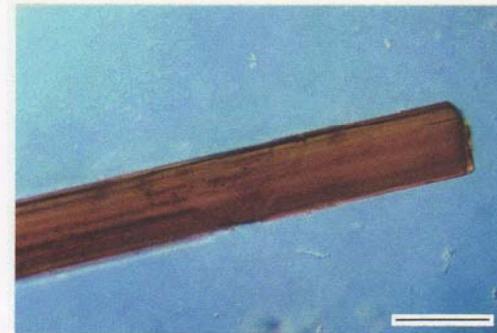
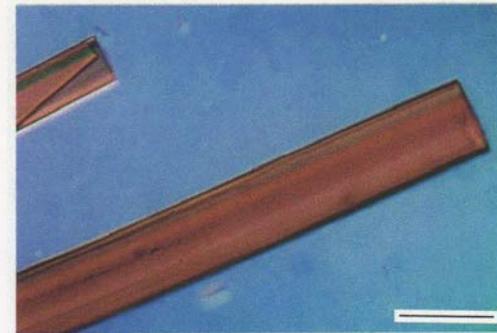


Fig. 9 The upper crystal is a Bacteriorhodopsin crystal grown in DCAM on Mir Increment 7. The lower crystal is from the DCAM ground controls illustrating typical defects and striations.



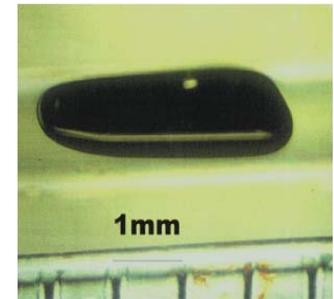
# Examples of Microgravity Grown Crystals

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## Past Success Stories: (2 examples from list of more than 30)

Example 1. Photosystem-I: Microgravity-grown crystals yielded dramatic improvement in crystal quality enabling structure determination.

Excerpt from published article: *“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

Example 2. STS 95 produced best crystals of **human insulin: in the T6 form** that diffracted to the highest resolution to that date, diffracting to 0.9 Å.

Excerpt from Nobel laureate Dr. Herbert A. Hauptman (1985 Nobel Prize in Chemistry) via letter to the honorable Dave Minge, U.S. House of Representatives, 23 June, 1995: *“I was initially quite skeptical about the possible benefits that might come from these efforts but I have clearly seen the advantages that this unique environment has to offer in the area of protein crystal growth”*