Commercial Protein Crystal Growth (C-PCG) Services on the ISS

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Executive Director, JSF

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Concept of C-PCG

- User satisfaction
- User friendly
- Highly expectable
- Shorter Preparation Duration
- Easier optimization
- Highly reliable & robust
- Lower cost

With maximizing μG effect

CS/MF/JSF has organized integrated system from a device to a standard service in collaboration with JAXA.

Since 2002, over 500 proteins have been tested on the ISS with the Japanese PCG hardware and processes. These processes go through continuous improvement to yield the highest probability of successful crystal growth, including a rigorous flight optimization of crystallization conditions.

CS/MF/JSF will provide researchers with access to Japan’s proven PCG technology.
Advantages of C-PCG Team and Processes

• Over 500 proteins tested on the ISS with the Japanese PCG processes and hardware since 2002
• Can manage several users and many proteins at the same time
• Focus is on providing **TOTAL SERVICES** of protein structure analysis to users
• The JCB-SGT (Japanese Crystallization Box – Sealed Gel Tube) is simple, reliable, and low-cost, with excellent scalability from terrestrial labs to ISS
• Initial acceptance testing of protein samples done to ensure quality and improve prospects for success of science on ISS
• Optimization of the crystallization conditions is done before launch
• JCB-SGT is passive (requires no crew time to start/stop)
• X-ray data collection is included as a standard service
• A complete protein structure analysis is provided to users (optional)
Possible Structure for a C-PCG Mission

Legend:
- Solid line: Contractual relationship
- Dotted line: Service/coordination
- Dashed line: Samples

Customers

JAXA / JEM-ISS

• Launch Opportunities
• KIBO Utilization

CS/MF/JSF
# Timetable of a C-PCG mission

## Standard Services

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<tr>
<th>Phase</th>
<th>Timing (Months)</th>
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## Option

- **(option) Further Purification**
- **(option) Crystal Quality Checked by Diffraction**
- **(option) Support user data collection in US**
- **(option) Phasing and Structure Determination**
- **(option) Refinement**
Standard services of C-PCG

Acceptance test
- SDS-PAGE
- Native-PAGE
- DLS
- Ion exchange chromatography
- 2-D PAGE

Optimization of crystallization condition for space experiment
- Counter-diffusion method
- Vapor-diffusion method

Crystal growth in space
- L-PGDS crystal grown in space diffracted up to 0.95 Å

X-ray diffraction data collection
- Crystal harvest and cryocooling service
- Progress in Baikonur
- Diamond light source
- A lot of experience of high-resolution X-ray diffraction data collection
- On-site data collection service

ISO 9001:2008 Certified
Crystallization Devices
- Counter-Diffusion Method -

Simple, Reliable, Light, Inexpensive, High-density, Without marangoni convection

http://lec.ugr.es/
Devices

- JCB-SGT 3-cell type
- JCB-SGT 6-cell type
- JCB main body with lid
- JCB-SLC for large crystals
- JCB-SGT stand
- A capillary, gel-tube and sealing compound
- Impulse sealer with sealer stand

- Capillary with gel-tube
- Place capillaries in the cell
- Seal JCB-SGT with the sealer
- Two JCB-SGT in the JCB main body

Load precipitant solution in the cell
A Success Story of Cosmic Drugging on ISS
Drug Therapy for Duchenne Muscular Dystrophy

University of TSUKUBA
Dr. Yoshihiro URADE
Duchenne muscular dystrophy (DMD)

- The most common form of muscular dystrophy that occurs in 1 out of 3,500 boys.

- DMD is caused by mutations of the dystrophin gene leading to low or no production of the cytoskeletal protein “dystrophin”.

Courtesy of Dr. Ikuya Nonaka
X-ray Crystallography of Hematopoietic Prostaglandin D Synthase (HPGDS)

Resolution

- Rat HPGDS 2.3 Å (Kanaoka et al., *Cell* 1997)
- Human HPGDS 1.7 Å (Inoue et al., *Nat Struct Biol.* 2003)
- Human HPGDS/TC-1 1.0 Å (Takahashi et al., *Acta Crystal.* 2010)
2.8 Å resolution

2.0 Å resolution

1.5 Å resolution

1.28 Å resolution
Protein crystallization under microgravity condition on International Space Station (ISS)
Chronic treatment of DMD dogs with HPGDS inhibitor

Dog A: TC-1 (10 mg/kg/day)

Dog B: Vehicle

Running test at 0 and 3 months, Running test at 11 months (months old)
Before treatment
Inhibitor

Vehicle
After 11 months
Vehicle
Inhibitor
Conclusion

HPGDS inhibitors are potential drugs used for DMD patients.
The Important Factors for All Field

(1) Cyclic on-board experiment
   → Improvement of experiment process and system
   → Production of important result

(2) International cooperation for large scale research to obtain significant results to contribute the human welfare.
Contribution to Life Innovation and Green Innovation.

The examples of the data obtained by protein crystal growth experiments on “Kibo”. They will contribute to various drug design and enzyme development useful for ecology.

Results obtained
• Development of a drug against muscle disease
• Structural data of protein related to the cancer cell.
On-going experiment
• Protein related to influenza virus, parasite disease

Resolution of protein structure: 0.96 Å
Bio-energy production from non-grain plants

Resolution of protein structure: 1.15 Å

Resolution of protein structure: 0.84 Å
Protein related to nylon resolution
Protein for new antibiotic drug design
Confocal Science Inc. (CS) / Maruwa Foods and Biosciences Inc. (MF)

• CS was founded in 1994 as an integrated service company for protein crystallography
• Experts in growing atomic-resolution crystals of target proteins for 3-D structural analysis since 2003
• Provides atomic-resolution protein crystallography service both in terrestrial and microgravity environment in cooperation with Maruwa Foods and Biosciences Inc., which offers cutting-edge technology for protein crystallization
• [http://www.confsci.co.jp/index_e.html](http://www.confsci.co.jp/index_e.html)
Japan Space Forum (JSF)

- JSF was established in 1994 as a non-profit organization to contribute to the enhancement of Japan's aerospace, science and technology by promoting businesses utilizing them
- Provides sponsorship and promotion of international conferences and symposiums
- Promotes programs to educate and enlighten the public about the aerospace, science and technology, as well as to fostering human resources in these fields
- Participates in space experiment support in the fields of materials and physical science, life science, space medicine and bio-technology
- [http://www.jsforum.or.jp/en](http://www.jsforum.or.jp/en)