# High-quality Protein Crystallization in Space

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# Abstract of JAXA project

- JAXA (Japan Aerospace Exploration Agency) has developed and established ultra-high quality protein crystallization technology using microgravity environments in JAXA-GCF project.
- High-quality protein crystals were grown based on technical improvements such as
  - Gel-tube method*1
  - 1-dimensional simulation
  - Techniques of harvesting and cryoprotecting crystals for X-ray diffraction experiments
- These technologies were shown in
  - Space-grown alpha-amylase crystal diffracted beyond 0.89 Å.
  - Of the protein that had high purity and succeeded in the optimization for space experiment, approximately 70% proteins improved in resolution.
- Growing high-quality protein crystals in space can help better understand 3-dimensional protein structure.
- The crystallization technology was originally developed by ESA (European Space Agency) and Prof. Garcia-Ruiz of Granada University.



- *1 Tanaka H. et al., J. Synchrotron Rad. (2004). 11, 45-48

# JAXA-GCF Space Experiment

| Mission | Odissea*1 | NASDA-GCF#1 | NASDA-GCF#2 | JAXA-GCF#3 | JAXA-GCF#4 | JAXA-GCF#5 | JAXA-GCF#6 |
|---|---|---|---|---|---|---|---|
| Launch | 25/09/2002 | 02/02/2003 | 29/08/2003 | 29/01/2004 | 11/08/2004 | 28/02/2005 | 21/12/2005 |
| at | Baikonur (Kazakhstan) | | | | | | |
| Vehicle | Progress | | | | | | |
| Landing | 07/12/2002 | 03/05/2003 | 28/10/2003 | 29/04/2004 | 24/10/2004 | 25/04/2005 | 01/04/2006 |
| at | USA | Kazakhstan | | | | | |
| Vehicle | Space Shuttle | Soyuz | | | | | |
| Duration | 10 weeks | 13 weeks | 9 weeks | 13 weeks | 9 weeks | 8 weeks | 14 weeks |
| Flight Facility | Granada Crystallization Facility (GCF) | | | | GCF and JCF (JAXA Crystallization Facility) | GCF (Vacuum insulator type) | GCF (Vacuum insulator type) |
| Number of GCBs (No. of protein samples) | 2 GCB (2 protein) | 46 GCB (36 protein) | 69 GCB (53 protein) | 50 GCB (41 protein) | 28 GCB-GT 9 GCB-HD (38 protein) | 39 GCB-GT 3 GCB-HD (36 protein) | 34 GCB-GT 11 JCB-HD (42 protein) |
| Installation Location | | Russian Service Module | CGBA (US module) | CGBA (US module) | TBU/Cryogem-3M (Russian Service Module) | TBU (Russian Service Module) | TBU (Russian Service Module) |

*1: ESA/Belgian Space Experiment

# Points of Technical Improvement (1/4)

- **Gel-tube method:** Crystallization device of Counter Diffusion (CD) newly developed by using a tiny gel tube with a high density container, based on Granada Crystallization Box. *1
- **Pre-flight procedures:** Standardized procedures such as a proper quality check and an optimization of sample.
- **Post flight treatments:** Crystal harvest from the capillary, cryo-protection and X-ray diffraction data collection at the synchrotron facility.

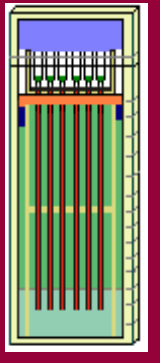
→ **These improvements are indispensable in improving our success rate.**



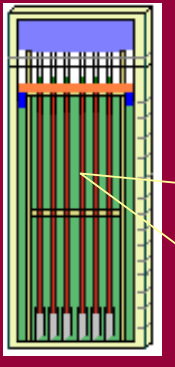
*1: Garcia-Ruiz, J.M., Moreno, A.:*Acta Cryst.*, D50, 484-490 (1994)

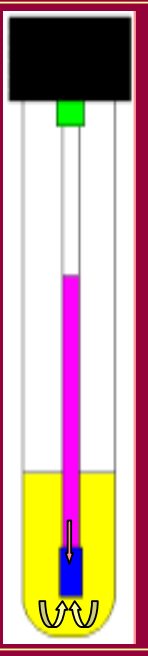
# Points of Technical Improvement (2/4)
## Crystallization Device

Gel Acupuncture method
(original)



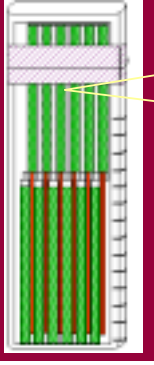
Gel-tube method





Granada
Crystallization Facility

JCB method
(gel-tube, high-density)



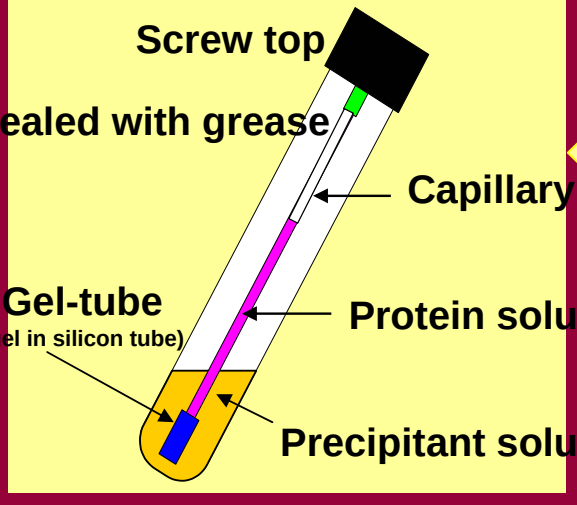
# Points of Technical Improvement (3/4)
# Pre-flight

Optimization of crystallization condition suited for space experiment

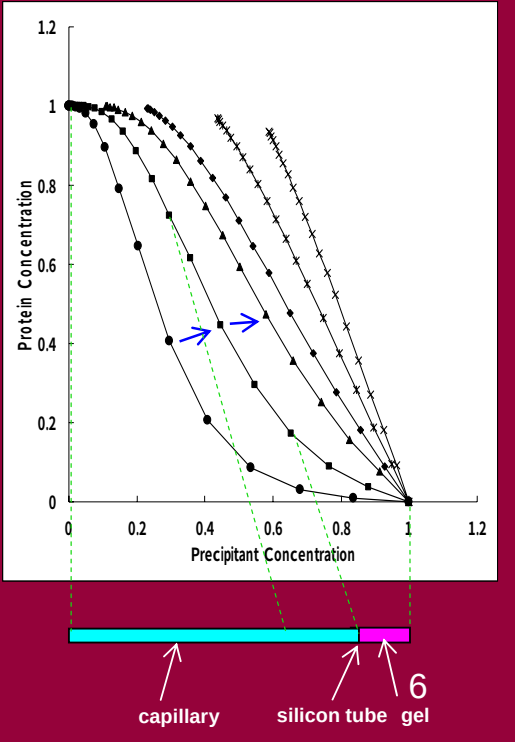
**Vapor-diffusion**

Preliminary experiment for optimization

1-dimensional simulation




**Crystallization condition could be converted from the vapor-diffusion method to the counter-diffusion method properly in a short period.**




**Counter-diffusion**

# Points of Technical Improvement (4/4)
# Post flight

Technical supports of harvesting and cryoprotecting crystals

Development of cryoprotectant database


http://idb.exst.jaxa.jp/

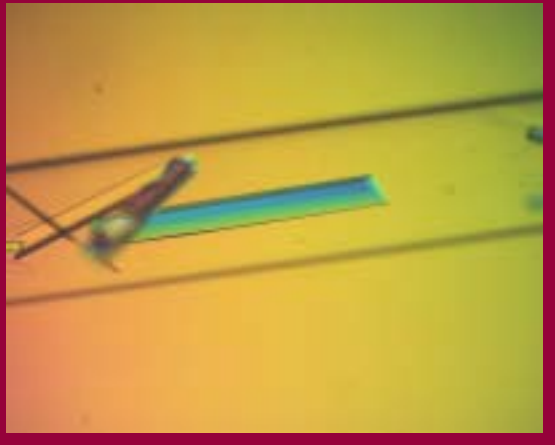
Development of crystal harvesting technique

A lot of X-ray diffraction data could be successfully collected.

# Typical example of space experiment (Alpha-Amylase (1))

Ground-grown

- Cluster-like formation
- Maximum resolution was 1.12Å (seeding)



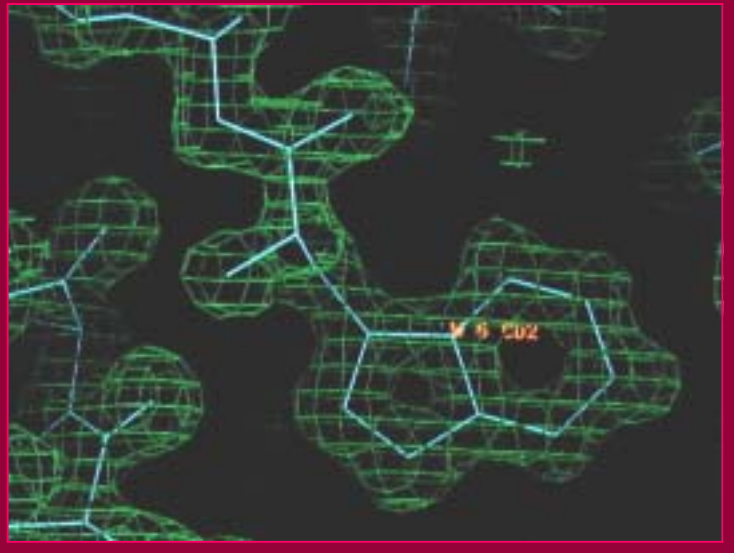
JAXA-GCF#1

Odissea mission

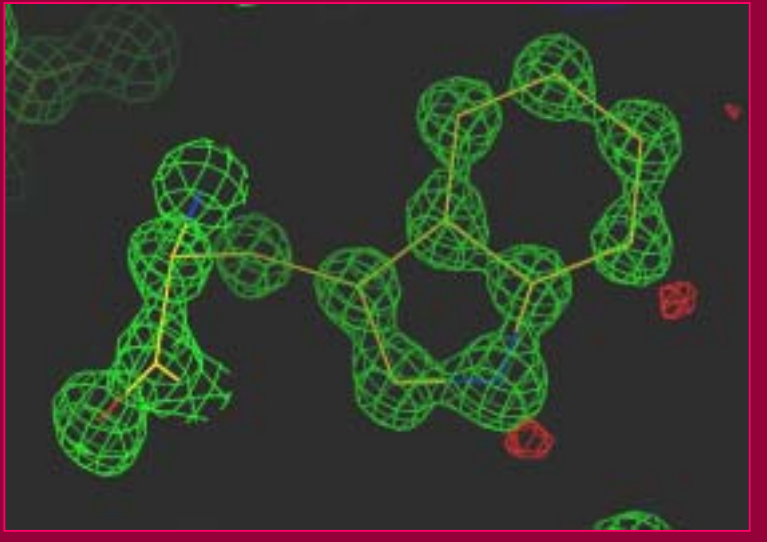
- Suppression of cluster-like formation
- Same morphology as ground-grown crsytal
- Diffracted beyond 0.89Å
- Different morphology in different space experiment

Protein solution： 90mg/ml alpha-amylase、100mM acetate buffer pH6.0
Precipitant solution： 40% PEG 8000、100mM acetate buffer pH6.0

# Typical example of space experiment (Alpha-Amylase (2))
## Electron Density Map



Crystal grown on the ground



Crystal grown in space

Atomic resolution structure can provide
- clear density map
- visualized hydrogen atoms
- visualized multiple conformations
- visualized unknown sugar chain

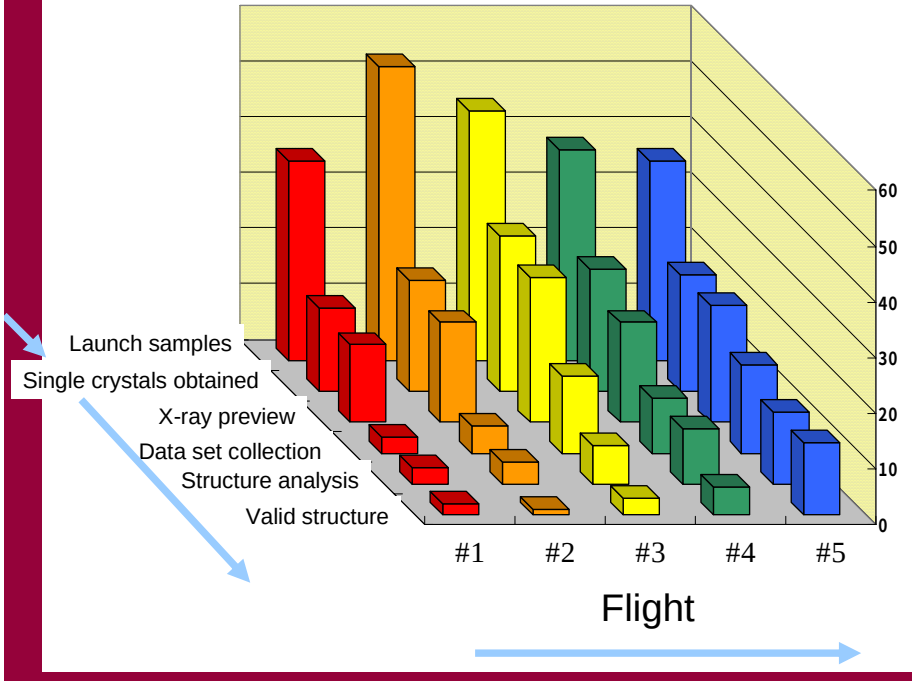
Data collected at SPring-8 BL12B2

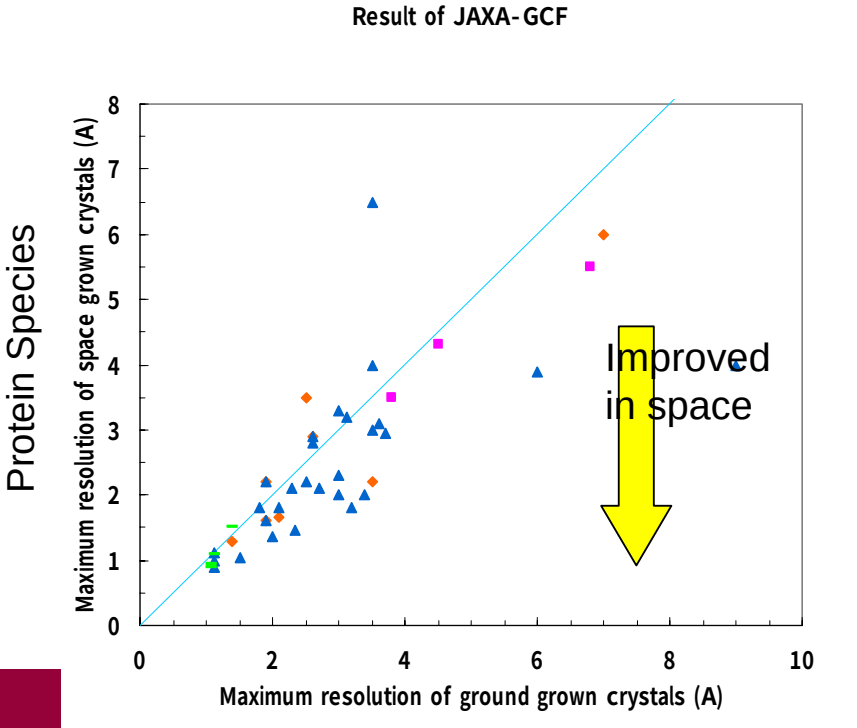
# Crystal Data and X-ray Data Processing Statistics (Alpha-Amylase (3))

| | 6TAA (Swift et al.*) | Ground experiment (Batch / Seeding) | Space experiment (GCB)** | Space experiment (GCB) |
|---|---|---|---|---|
| X-ray source | Conventional | SPring-8 BL12B2 | SPring-8 BL12B2 | |
| Wavelength (Å) | | 1 | 0.7 | |
| Space Group | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ | |
| Cell constant (Å) | a=51.0, b=67.2, c=133.6 $\alpha=\beta=\gamma=90^o$ | a=50.8, b=67.7, c=130.1 $\alpha=\beta=\gamma=90^o$ | a=50.4, b=67.4, c=130.4 $\alpha=\beta=\gamma=90^o$ | |
| Volume of the cell | 457,960 | 447,187 | 442,807 | |
| Maximum resolution (Å) | 2.1 | 1.12 | 0.89 | |
| Mosaicity | | 0.312 (~1.4Å) | 0.241 (~0.9Å) | |
| Average of $I/\sigma$ (I) | | 22.2 | 39.2 | 26.6 |
| Rmerge(overall) | | 0.063 (30-1.40 Å) | 0.036 (30-1.40 Å) | 0.062 (15-1.0 Å) |
| Rmerge(outer shell) | | 0.188 (1.45-1.40 Å) | 0.039 (1.45-1.40 Å) | 0.262 (1.04-1.00 Å) |
| Completeness (overall) (%) | | 94.5 (30-1.40 Å) | 99.3 (30-1.40 Å) | 96.5 (15-1.0 Å) |
| Completeness (outer shell) (%) | | 89.9 (1.45-1.40 Å) | 99.3 (1.45-1.40 Å) | 95.7 (1.04-1.00 Å) |

*Swift, H.J. et al.:Acta Cryst., B47, 535-544 (1991)
**Calculated for the comparison with the data of the ground experiment.

# Improvement of the resolution




- Success rate became higher as flight experiment proceeded.
- In the case of PEG as a precipitant, maximum resolution of space-grown crystals was better than that of ground-grown crystals in many cases.

# Example of Improvement

| Quick Check & Optimization | Crystallization in Space | Diffraction Data Quality | Example of resolution improvement (Å) |
|---|---|---|---|
| Good: 20／26 | Diffraction quality obtained: 18／20 | Higher quality obtained: 13／18<br><br>Resolution improvement: 12／13<br>Mosaicity improvement: 1／13 | 4.5→4.3, 3.8→3.5, 3.7→2.9, 3 →2.3, 2.1→1.8, 2.1→1.7, 1.9→1.6, 1.9→1.6, 1.8→1.8, 1.5→1.1, 1.12→0.89,1.1→0.88 |
| | | Fail: 5／18 | |
| | Fail: 2／20 | N/A | |
| Fail: 6／26 | Diffraction quality obtained: 0／6 | N/A | |
| | Fail: 6／6 | N/A | |

**Of the protein that had high purity and succeeded in the optimization for space experiment, approximately 70% proteins improved in resolution.**

# Benefit of the Space Crystallization

- Improvement of the resolution from atomic level to subatomic level
- Improvement of the mosaicity
- Precise 3-D atomic coordinate
  - –Hydrogen atoms
  - –Protonation state
  - –Multiple Conformation

→ **Contribution to efficient drug discovery**

**Protein crystallography at subatomic resolution**

**Dr. T.Petrova and Dr. A. Podjarny**

**Department of Structural Biology, IGBMC**

**Reports on Progress in Physics 67 (2004) 1565-1605**

- Suppression of the cluster-like crystal formation
- Improvement of the twin crystal

# Acknowledgement


- ESA / Belgian government
- CSIC-University of Granada / Prof. García-Ruiz and the members of the laboratory
- Protein Sample Provider: Protein 3000 Project (Riken & 8 Universities), NIAS, PCProt, other users
- Russian Federal Space Agency and RSC Energia
- NASA
- SPring-8 (JASRI)
- IPR Osaka University / Prof. Nakagawa
- PharmAxess, JGC

# Ultra High Resolution Protein Crystallization Service in Space

By JSF group

# Background of privatization plan

- JSF (Japan Space Forum) became a successor of these technologies of the JAXA project (has conducted from 2002 to 2006) for privatization.
- JSF has organized a technical support team, which consists of four private sectors and one public-service foundation. JSF group has started an ultra-high quality protein crystallization service in space, using Russian space flight opportunity and the International Space Station. The group has also started useful data collection service using the synchrotron facility .

# Outline of Service

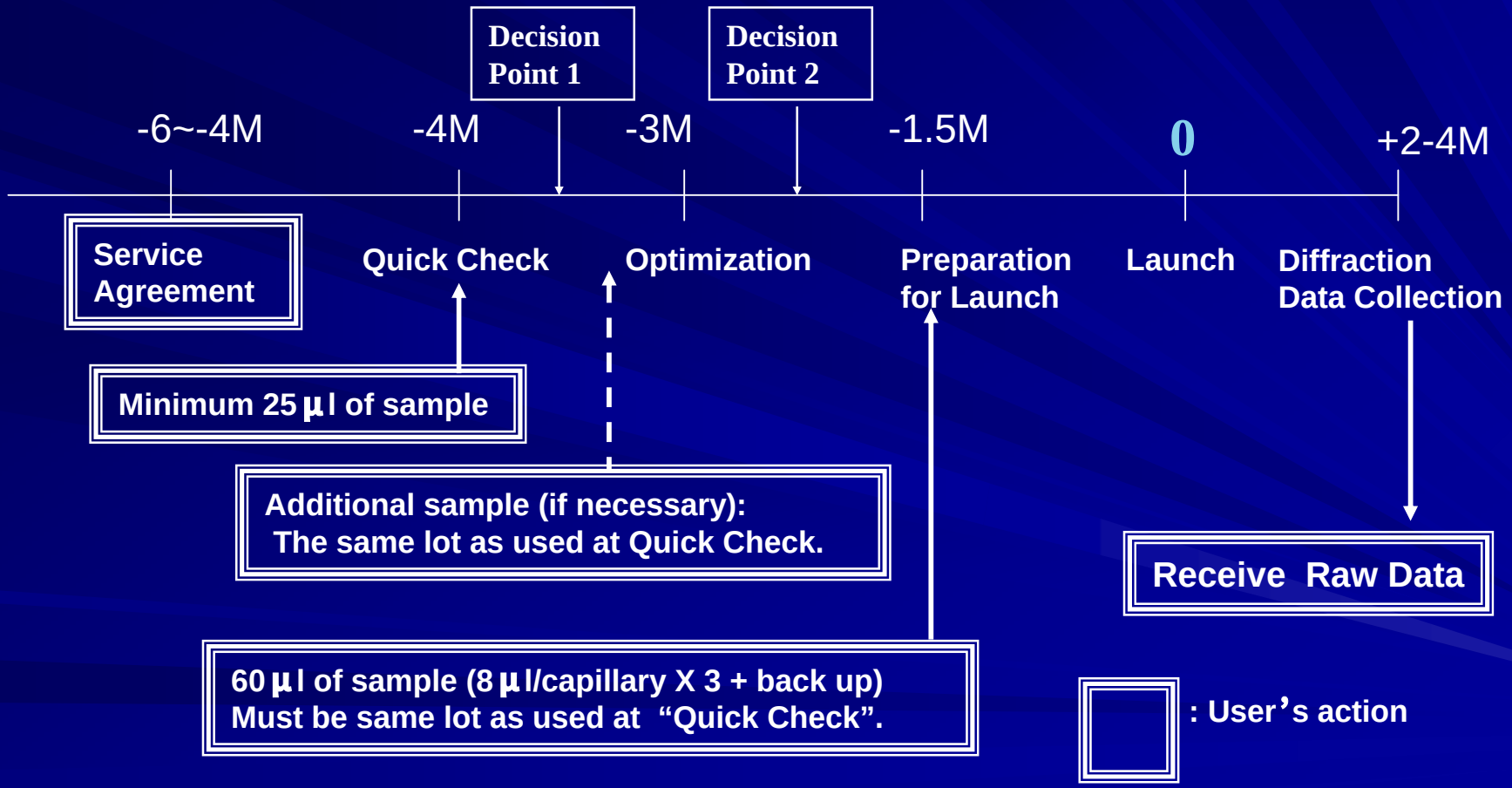
- JSF Group is going to provide opportunities on the Russian space flight program semiannually from the end of 2006.
- Standard service
  - Quick Check: Quality and crystallization check (VD or batch)
  - Optimization: Optimize for a space crystallization device (JCB–HD*1) and microgravity environment.
  - Documentation: Processing all the necessary documents for the ISS safety panel and sample transportation.
  - Crystallization in space :Using JCB-HD
  - Diffraction Data Collection: Post flight treatment and data collection at the synchrotron facility
- Optional service
  - Expression of the protein for the crystallization
  - Purification of the protein sample
  - Process raw data to F-data
  - Process F-data to electron density map
  - Provide or synthesize ligand compound

*1 JAXA Crystallization Box High density

# Expected Result for User

- **Improvement in resolution of launched protein is expected, if the protein provides high purity and succeeds in optimization for space experiment.**

# Procedure & Schedule



# Requests for users

Users are asked the following points about a protein sample and components of relevant solutions ;

- to guarantee
  - no toxic or pathogenic.
  - not controlled as the strategic materials by Japanese government.
- to disclose the necessary information to support our documentation process for the ISS safety panel and the Japanese and Russian customs.

  ex. Abstracts of the protein name

  Its biological function, etc.
- to disclose some necessary information, such as the detail of the crystallization processes in the user's lab, to expedite our experimental processes.

# Preservation of confidentiality

Non disclosure agreement will be arranged prior to the main contract.

The agreement will contain the following points.

- All the confidential information of the user will be strictly controlled.
- All the information obtained through our service belongs to the user and is strictly controlled except any information related to our crystallization technologies.

# JSF Group

- JSF group consists of the following members:
  - Japan Space Forum (JSF): Juridical public-service foundation, established in February 1994. One of the typical supporting bodies for the Japan Aerospace Exploration Agency, or JAXA.
  - Mol Logic Inc.: Operates biochemical and crystallographic exp.
  - Patcore Inc.: Provides chemical synthesis of ligand molecules.
  - Confocal Science Inc.: Supports space exp. optimization.
  - JGC Corp.: Interfaces with Russian partners.

**Contact point: proteincrystal@jsforum.or.jp**

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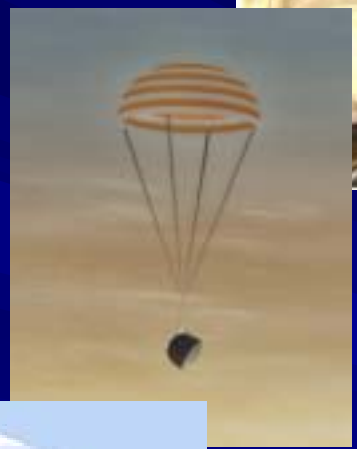

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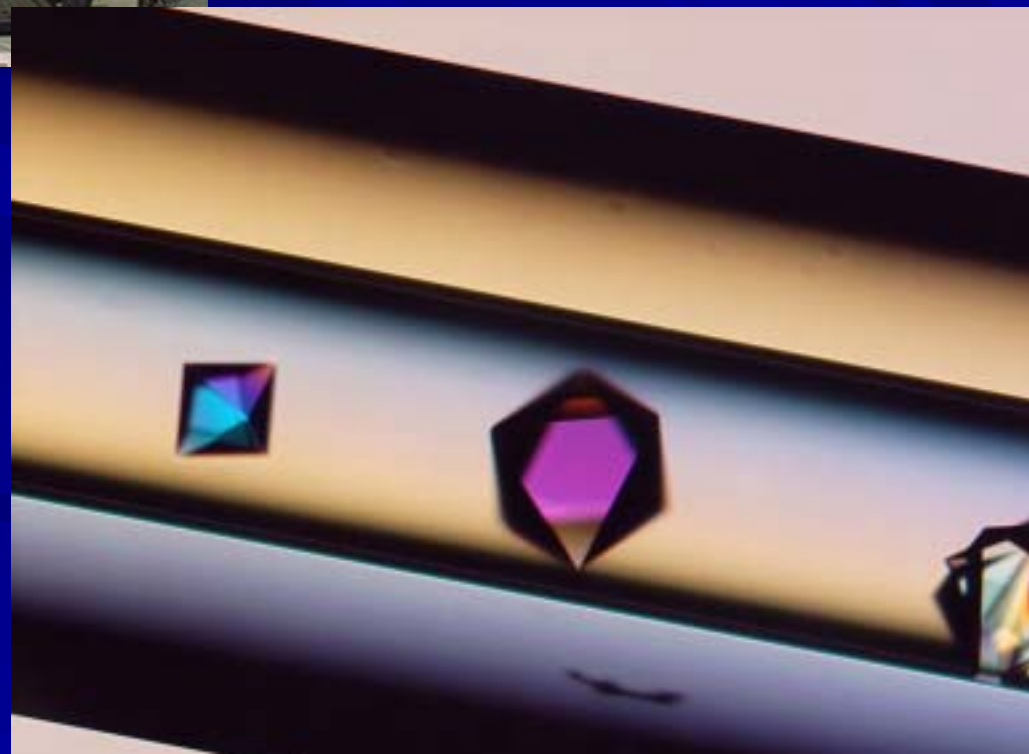

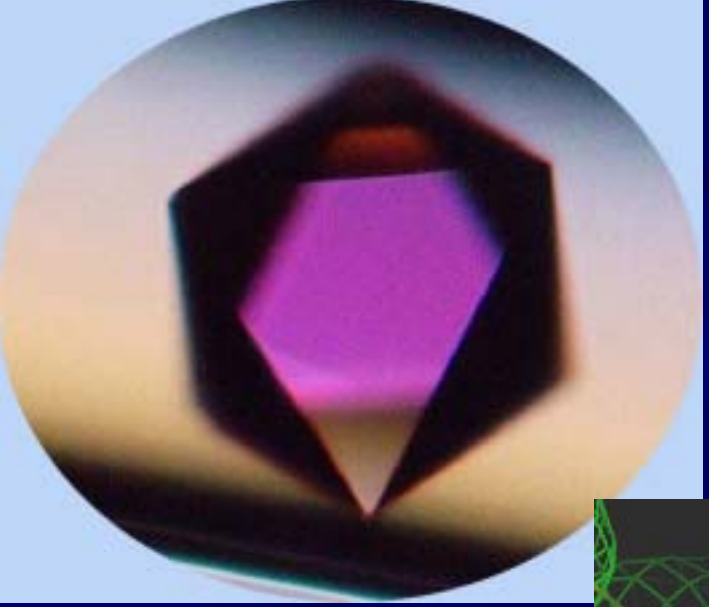

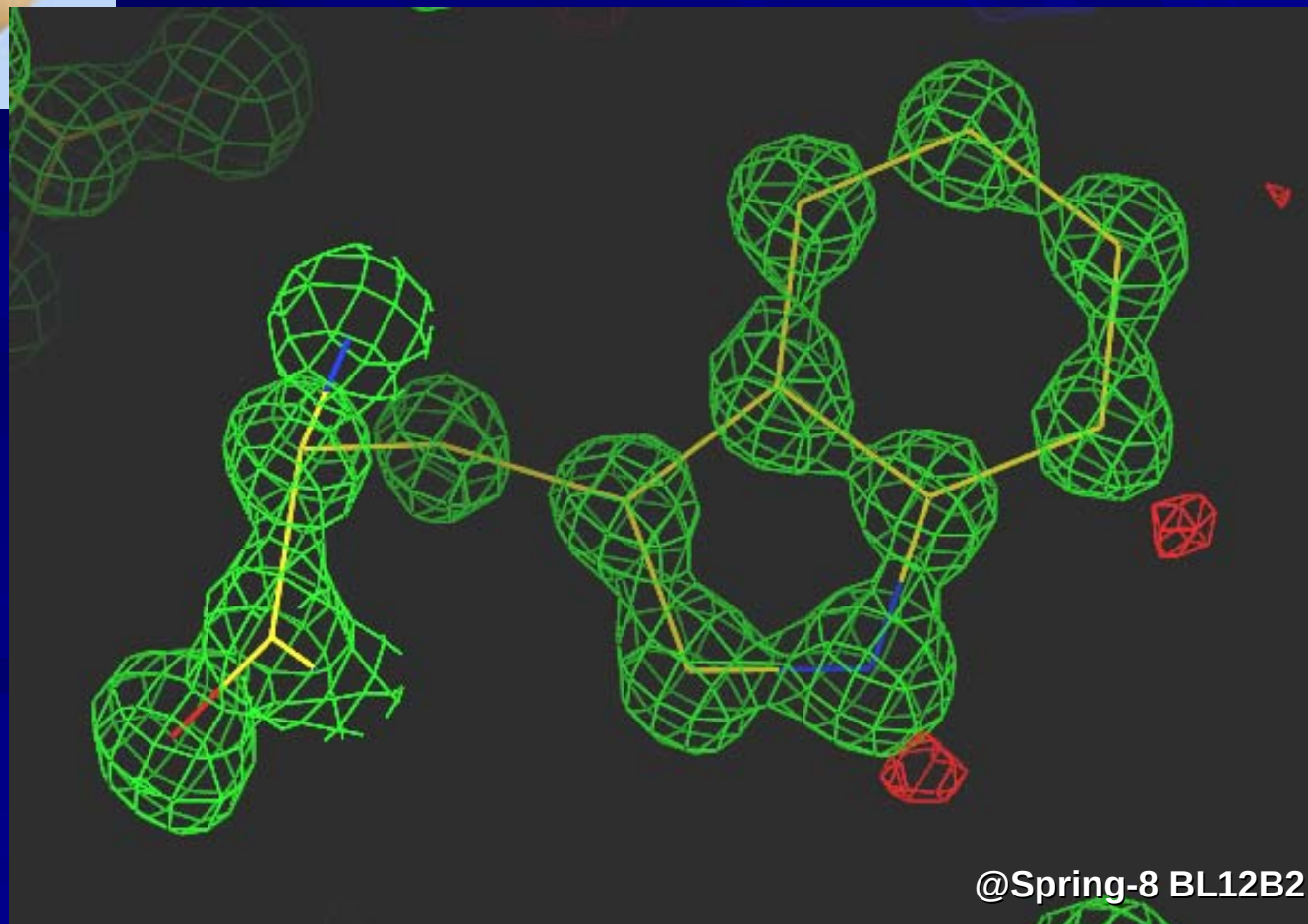
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