JAXA-GCF#4宇宙実験実施状況

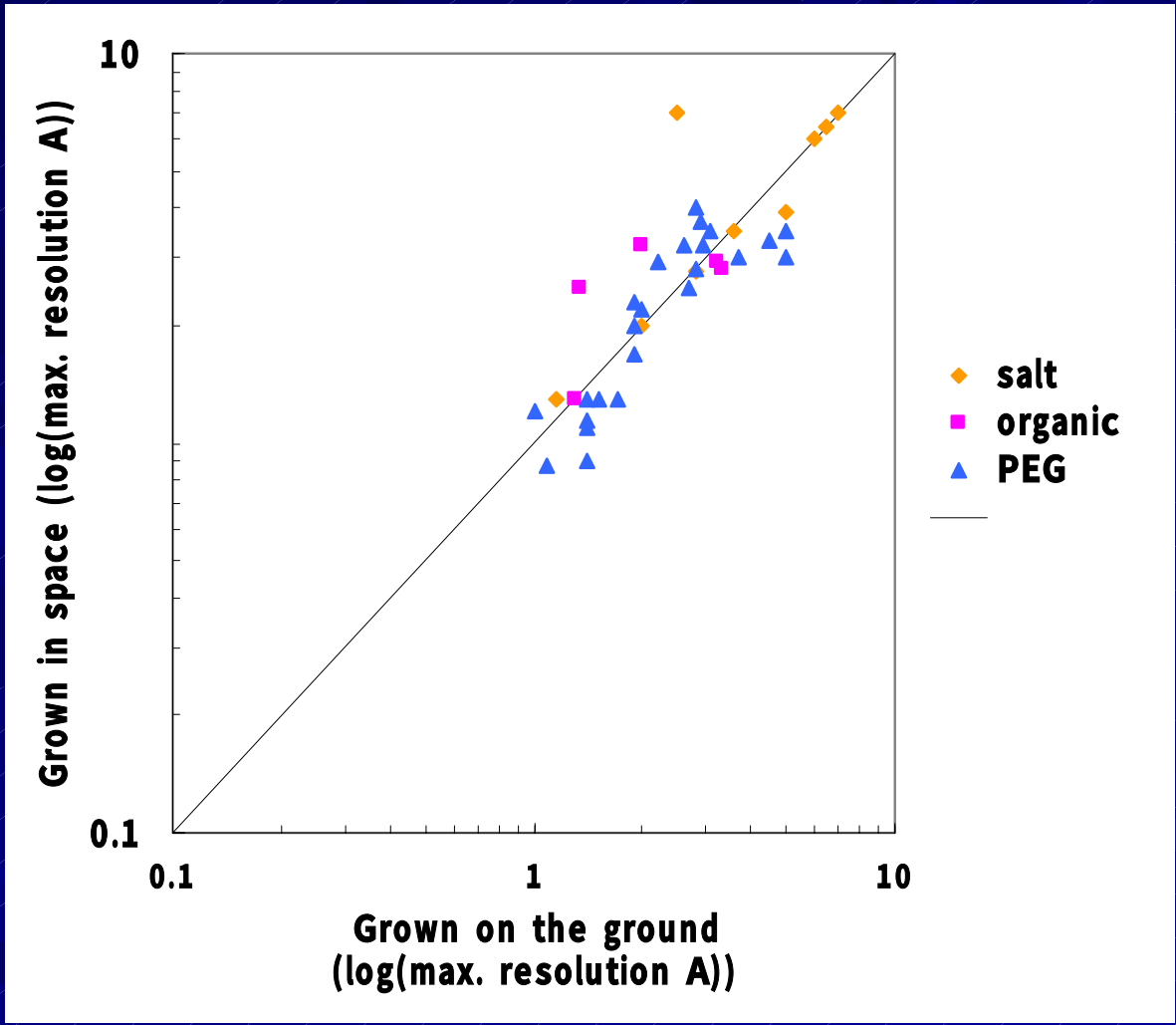
平成17年2月24日
財)宇宙環境利用推進センター

JAXA-GCF Space Experiment

Mission	Odissea	NASDA-GCF#1	NASDA-GCF#2	JAXA-GCF#3	JAXA-GCF#4	JAXA-GCF#5
Launch	25/09/2002	02/02/2003	29/08/2003	29/01/2004	11/08/2004	28/02/2005(TBD)
at	Baikonur (Kazakhstan)					
Vehicle	Progress					
Landing	07/12/2002	03/05/2003	28/10/2003	29/04/2004	24/10/2004	25/04/2005(TBD)
at	USA	Kazakhstan				
Spacecraft	Space Shuttle	Soyuz				
Duration	10 weeks	13 weeks	9 weeks	13 weeks	9 weeks	8 weeks
ght Facility	Granada Crystallization Facility (GCF)				GCF and JCF (JAXA Crystallization Facility)	GCF (Vacuum insulator type)
Number of GCBs (No. of rotein samples)	2 GCBs (2 proteins)	46 GCBs (36 proteins)	69 GCBs (53 proteins)	50 GCBs (41 proteins)	28GCB (GT method) Total 37GCBs (38 proteins)	36GCB (TBD)
tein for JAXA cal verification	2 proteins	1 protein	1 protein	2 proteins	6 proteins	TBD
Installation Location		Russian Service Module	CGBA (US module)	CGBA (US module)	TBU/Cryogem-3M (Russian Service Module)	TBU (Russian Service Module)

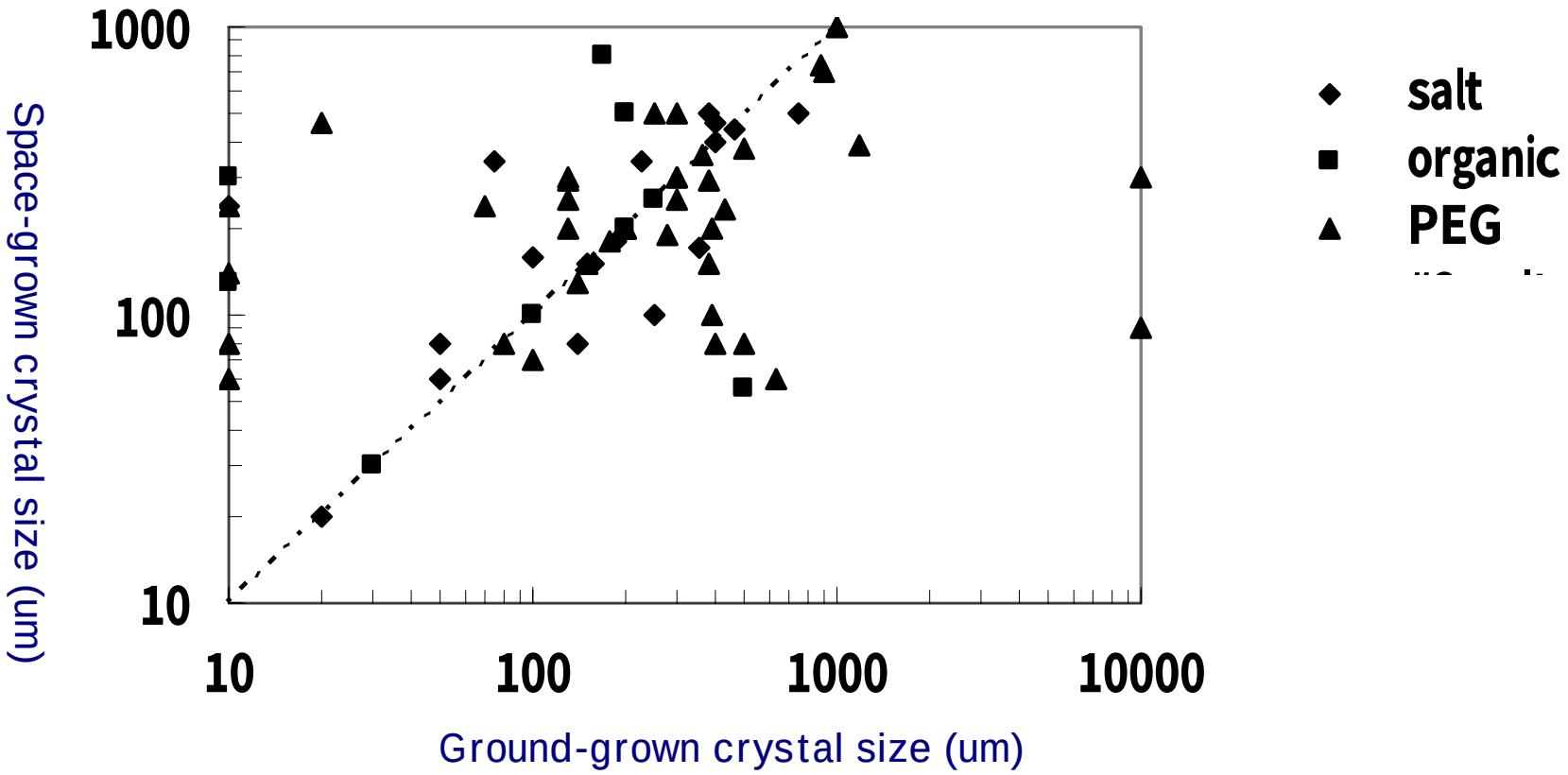
Results of #1~#3

Comparison of Maximum Resolution between Space- and Ground-grown crystals



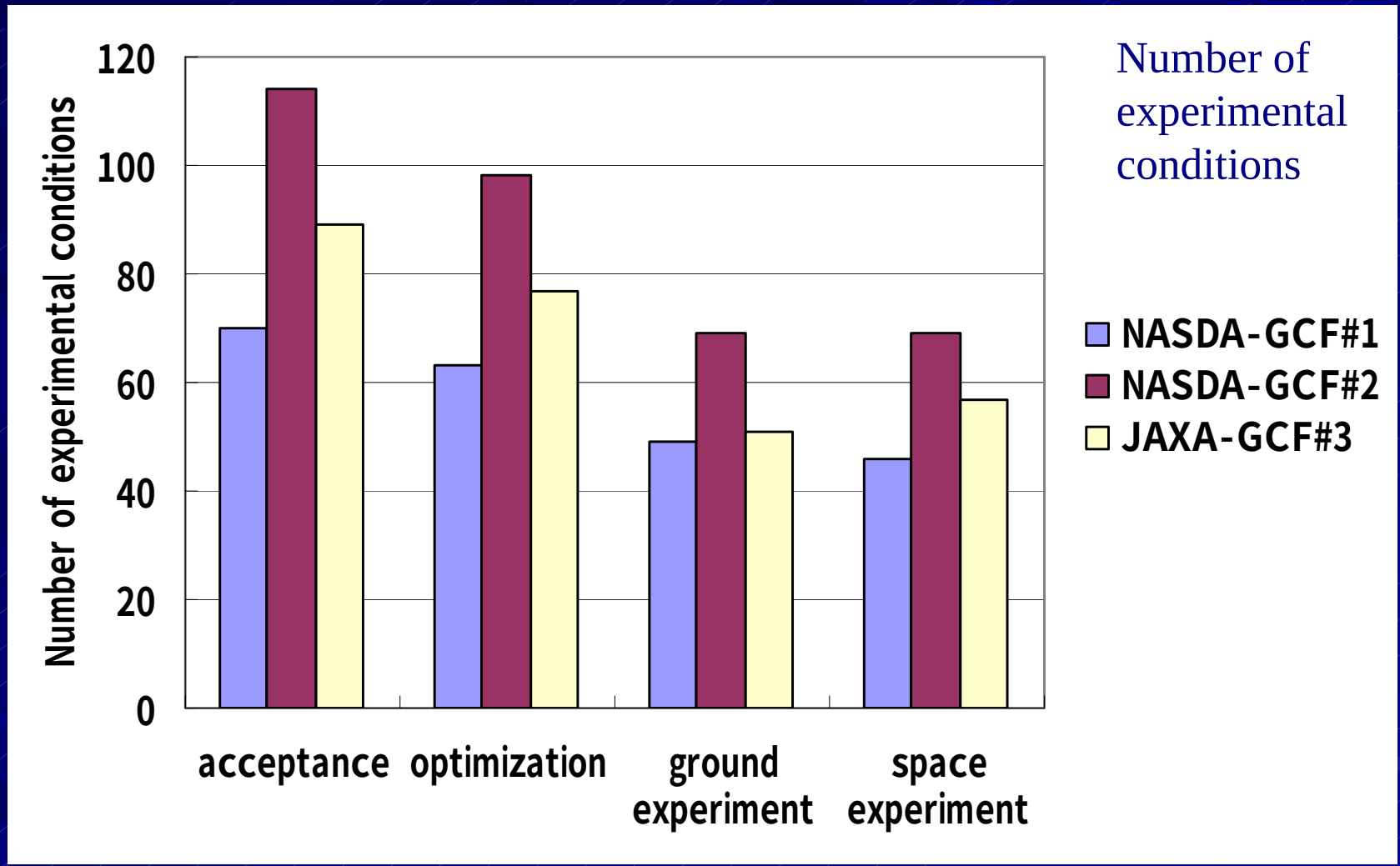
Data are collected from crystals grown in JAXA(NASDA)-GCF#1, #2, and #3 space experiments

Comparison of Crystal Size between Space- and Ground-grown crystals

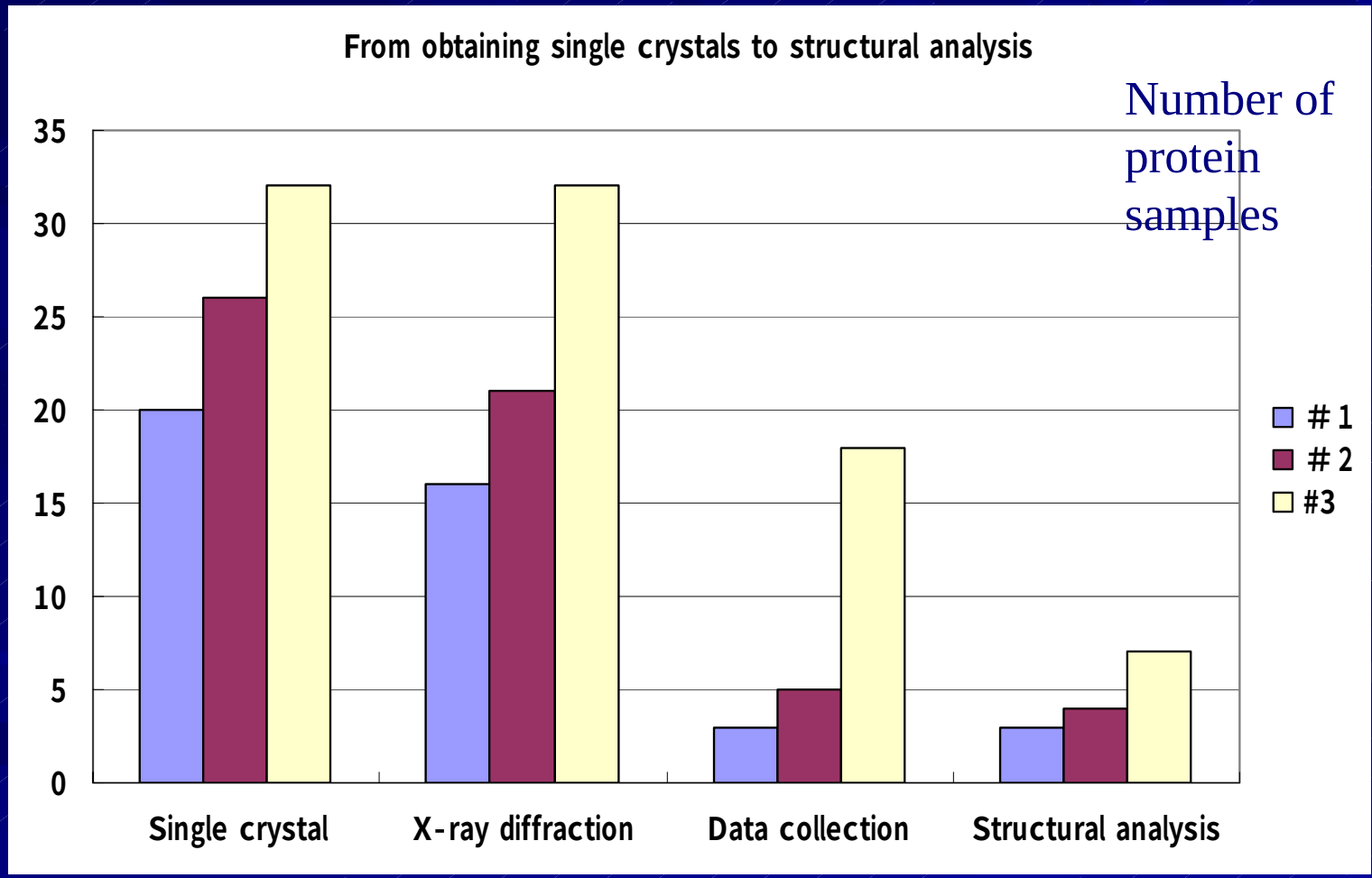


Data are collected from crystals grown in JAXA(NASDA)-GCF#1, #2, and #3 space experiments

From acceptance of samples to space experiment



From obtaining single crystals to structural analysis



JAXA-GCF#3: 2004.10.05

Tentative results of JAXA-GCF #4 Experiment

11/08/2004~24/10/2004

日程概要

- 利用者説明の実施（～3月）：済み
- データシートの受付（～３月１５日）：一次調整（～３月２０日）
- NIHSでの安全性評価：3月30日
- 試料送付依頼：試料受け渡し（1回目）（～４月１３日）
- 利用者からJSUPへ送付。試料番号を付けなおして委託業者へ
- 条件絞込み実験#1（4月１５日～5月15日）
- 試料性状検討（必要なものについて）（5月１５日～５月３０日））・・・35+7種。終了（適は13種）
- 条件絞込み実験#2（5月１５日～６月３０日））（13+αについて実施）
- 迅速対応の状況確認（6月20日）
- 搭載試料の決定（6月末）・・・○：32、△：11
- 試料送付依頼：試料受け渡し（2回目）（7月13日依頼、7月21、22日着）
- 時間経過調整（～7月16日）
- 射場作業（8月2日～11日）、打ち上げ（8月11日）
- 地上確認実験（8月23日より）
- 回収作業（１０月26日）

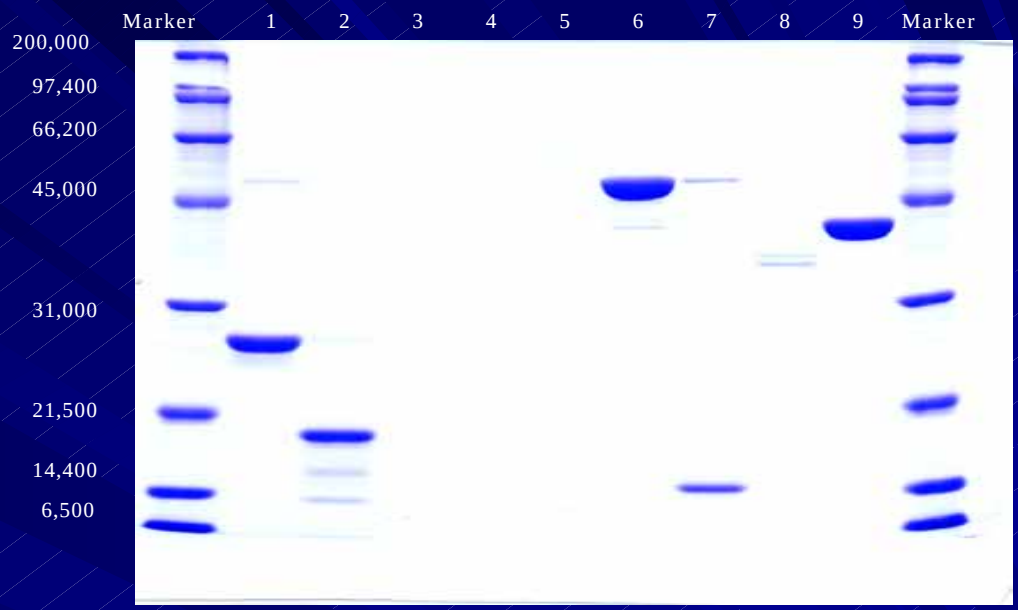
JAXA-GCF#4

What was special in this flight…

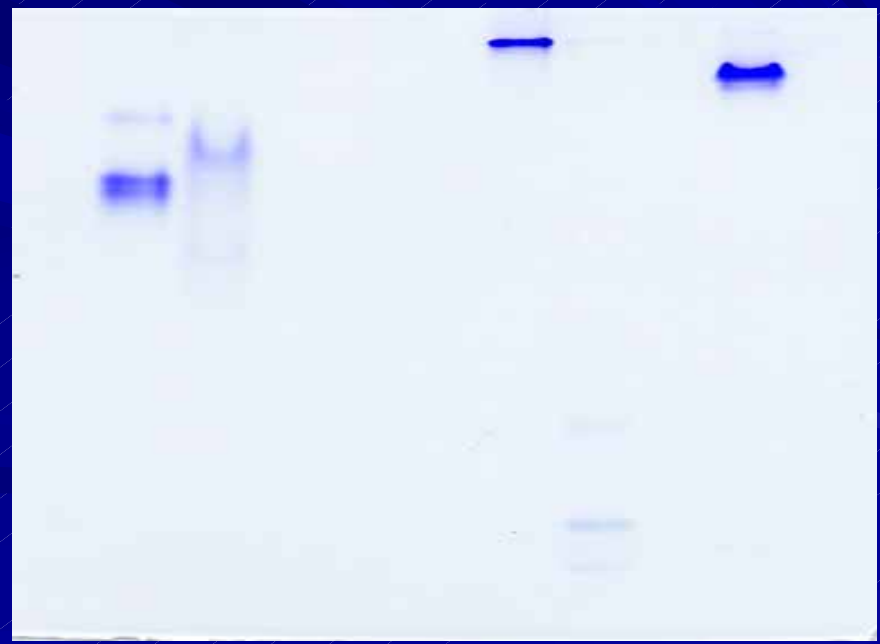
- 37 GCBs (38 proteins) were launched.
  - Users: 32 proteins
  - JAXA: 6 protein (alpha-Amylase, Lysozyme, etc.)
- For optimization of crystallization condition, gel-tube method and sample purity check were applied.
- PEG was recommended to add in precipitant solution.
- In case of salt or organic precipitant, PEG was added in 8 cases.
- For more stable temperature in ISS, alkan and vacuum insulator were packed in GCF to keep it 18~20C in addition to the usage of TBU/Cryogem 3M in Russian Service Module.
- Thermos bottle(JCF) was developed for temperature stability.
- JCB(JAXA crystallization box) was developed and applied for 9 GCBs.
- Technical supports of harvesting and cryoprotecting crystals were conducted by JAXA for 7 users out of 10 users.
- Some samples were loaded in Japan (7 proteins, 8 samples).
- All samples were crystallized in gel-tube method.

Sample Purity Check

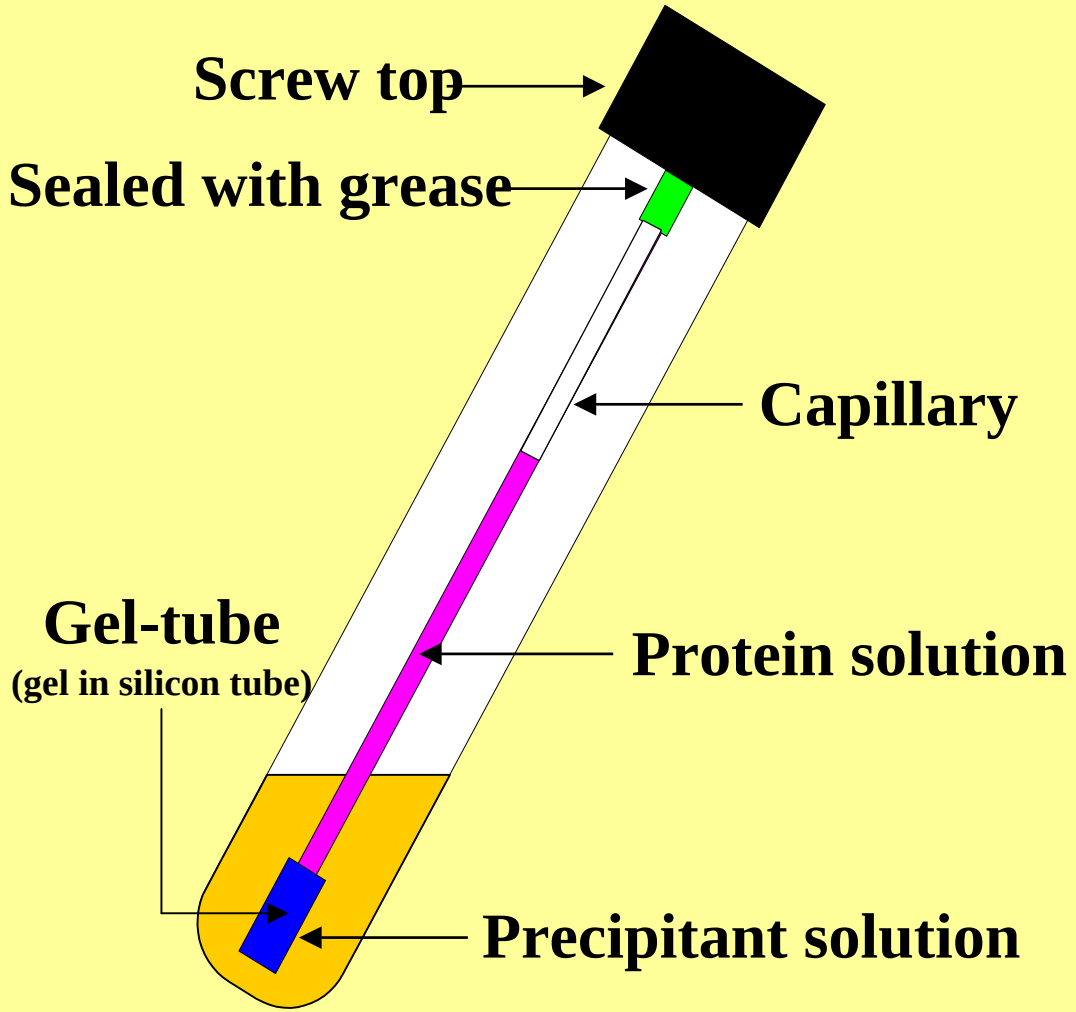
SDS-PAGE



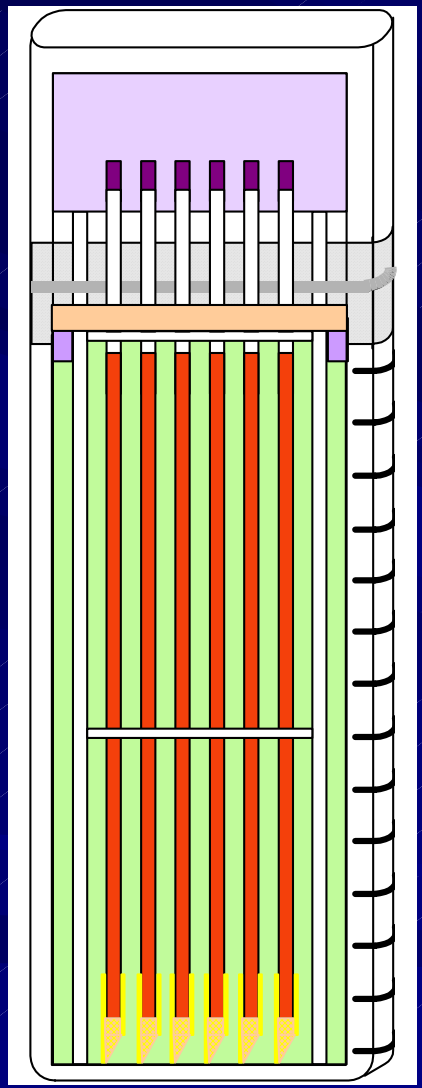
Native-PAGE



Outlook of a ‘Gel-Tube’ Crystallization Device

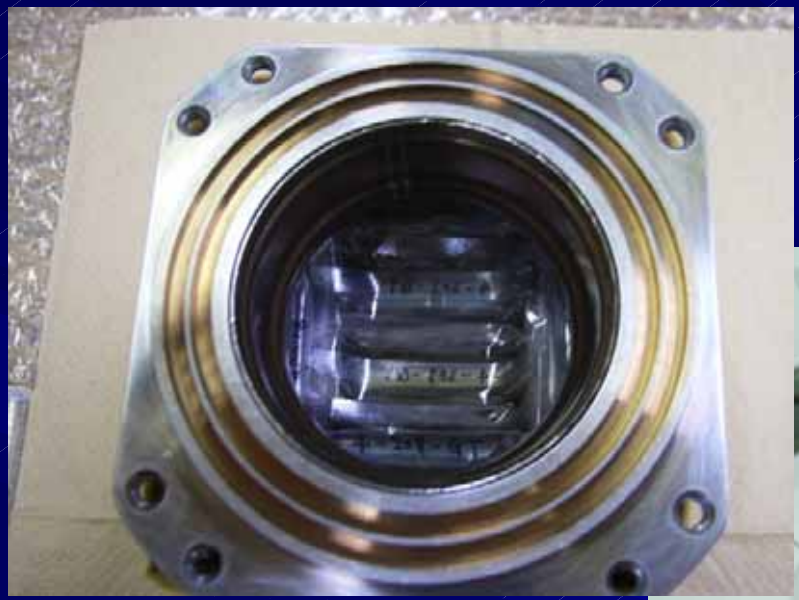


ゲルチューブ法のメリット



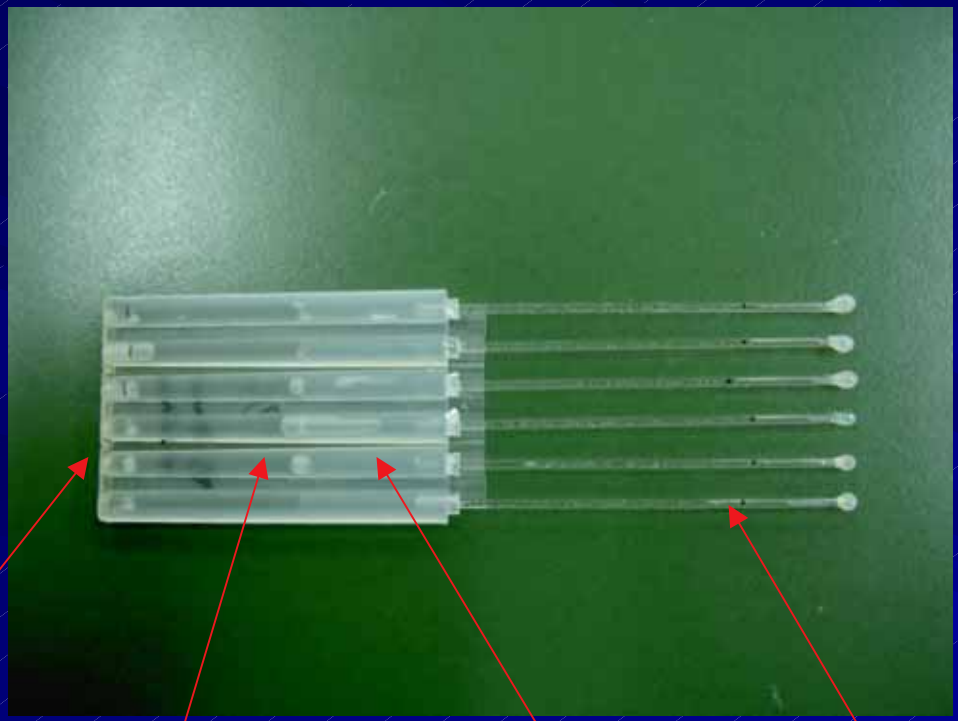
★拡散が比較的速い
★（溶液量が少なくてすむ）
★ゲル体積が小さいため溶液濃度が希釈されない
★キャピラリーからの液漏れが少ない
★シリコンチューブ入りゲルの使用でゲルの準備が簡便
★宇宙実験で利用
- #2フライト　20/69
- #3フライト　35/50
- #4フライト　74/74

Thermos Bottle(JCF)



JCB
(JAXA Crystallization Box)

Outlook of JCB

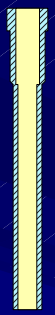


Features of JCB

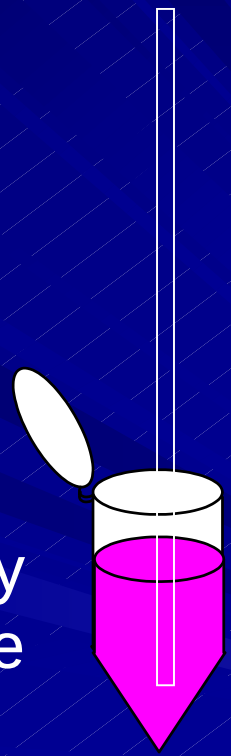
- JCB can be fit in Granada Crystallization Box (GCB).
- Gel-Tube method is applied.
- Precipitant solution is separated for each capillary.
- Injection molding is used for manufacturing JCB.
- Easy to change the Precipitant solution individually after fixing the capillary.

How to assemble JCB (1)

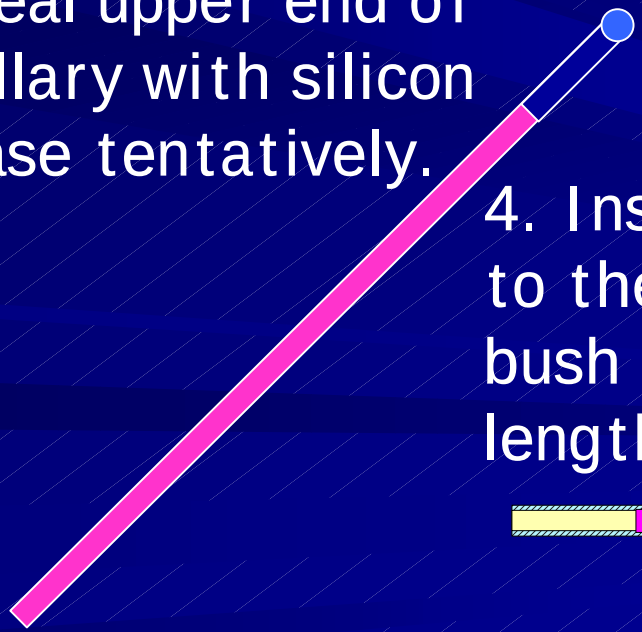
1. Prepare 1% agarose gel in bush.



2. Fill up capillary with protein solution.



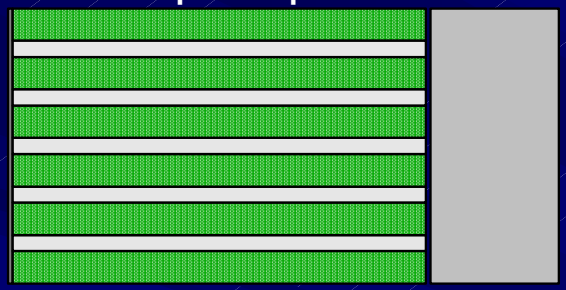
3. Seal upper end of capillary with silicon grease tentatively.



4. Insert glass capillary to the bush and cut the bush to appropriate length.

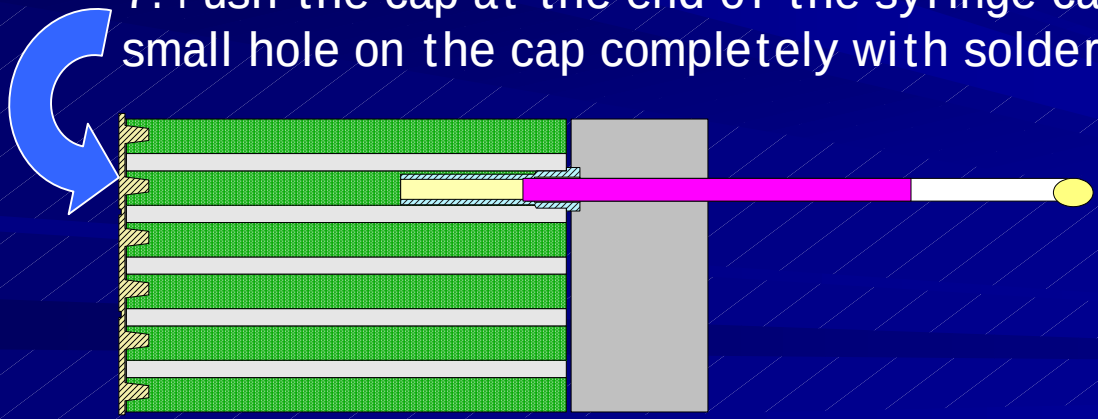
How to assemble JCB (2)

5. Pour precipitant solution into syringe case.



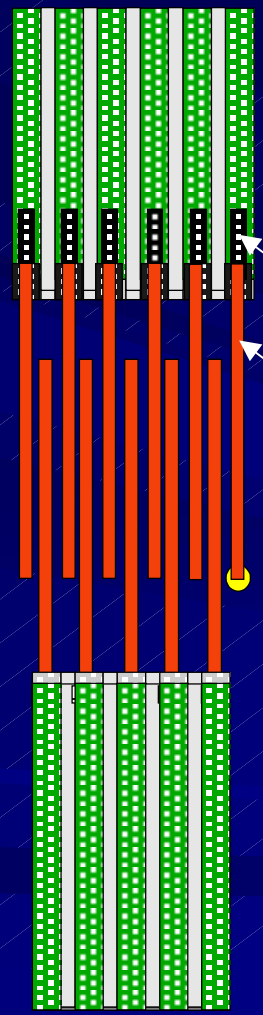
6. Install capillary into JCB syringe case.

7. Push the cap at the end of the syringe case and seal the small hole on the cap completely with soldering iron.



8. Seal the upper end of the capillary completely and the gap between the capillary and the bush.

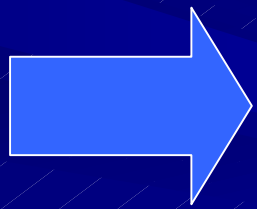
Two JCB syringe cases are installed in GCB case

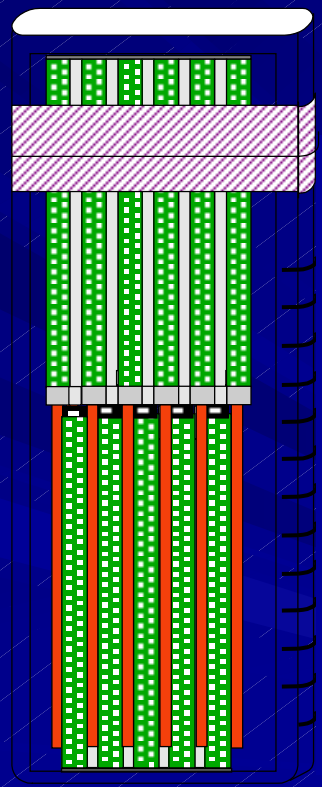


Two JCB syringe cases are combined
and installed into GCB case.

■ :Gel in bush

■ :Protein in the capillary





JCB in JAXA-GCF#4 Experiment

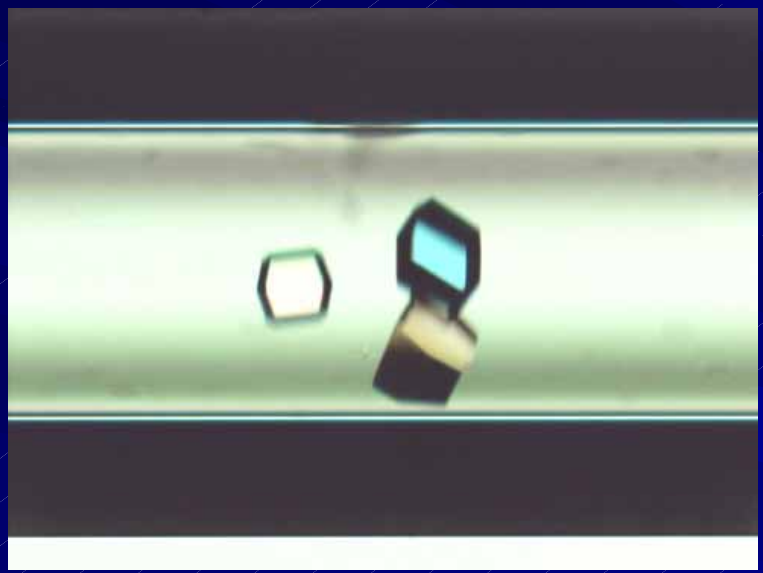
	GCB	*Protein*	*Crystallization Condition*
GT	*28*	*26*	*28*
JCB	*9*	*12*	*46*

Comparison of Crystal Evaluation between GCB and JCB

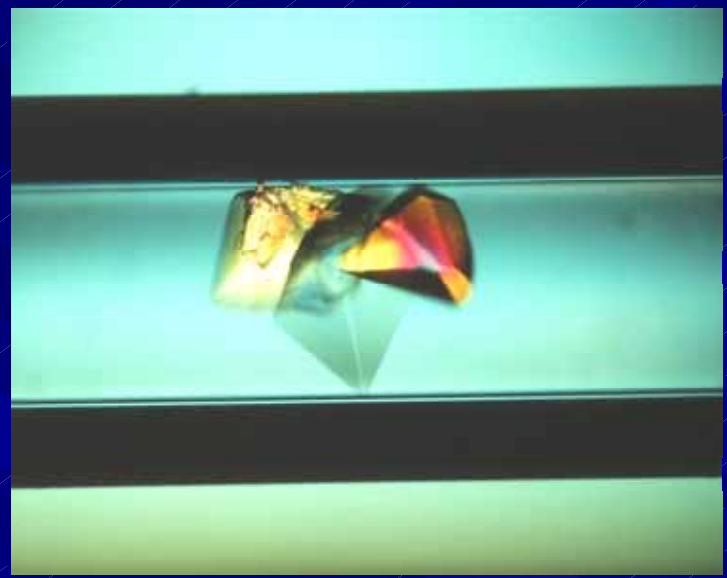
		JCB (crystal evaluation)			
		1	2	3	4
GT (crystal evaluation)	1	1	0	0	0
	2	0	0	0	0
	3	0	0	1	0
	4	0	0	2	10

Evaluation	1: no crystal	2: oil, precipitate
	3: insufficient crystal	4: single crystal

Lysozyme crystals in JCB



Grown in space



Grown on the ground

Critical Points

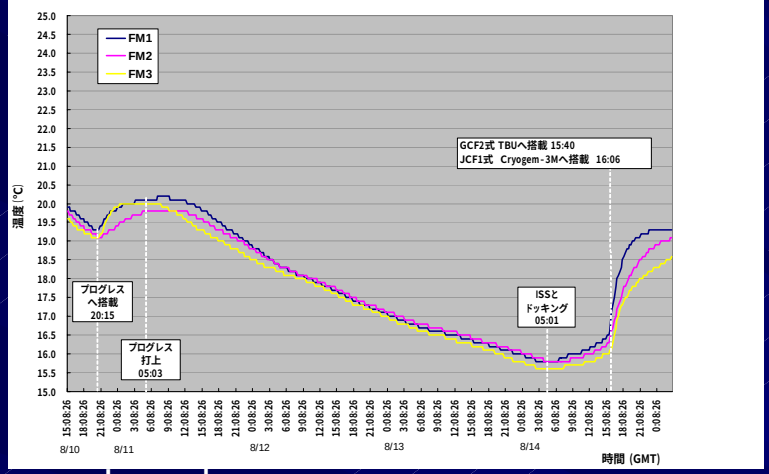
- JCB is still underdevelopment phase.
- There are several problems to be solved.
  - Leakage of precipitant solution
  - Crack of syringe case
  - Quality of the crystals are deviated.

JAXA Crystallization Box (JCB)

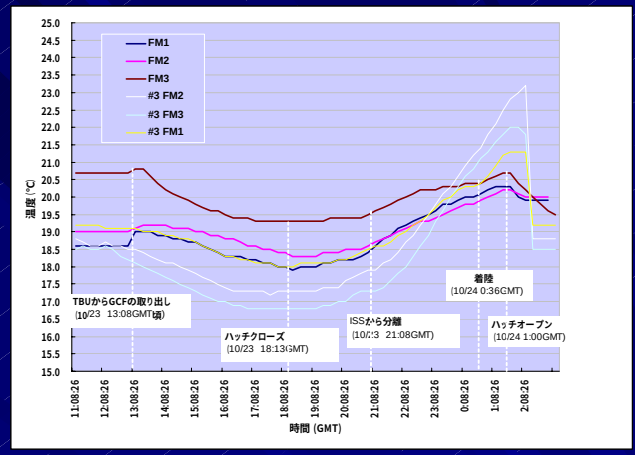
JAXA-GCF#4

Results

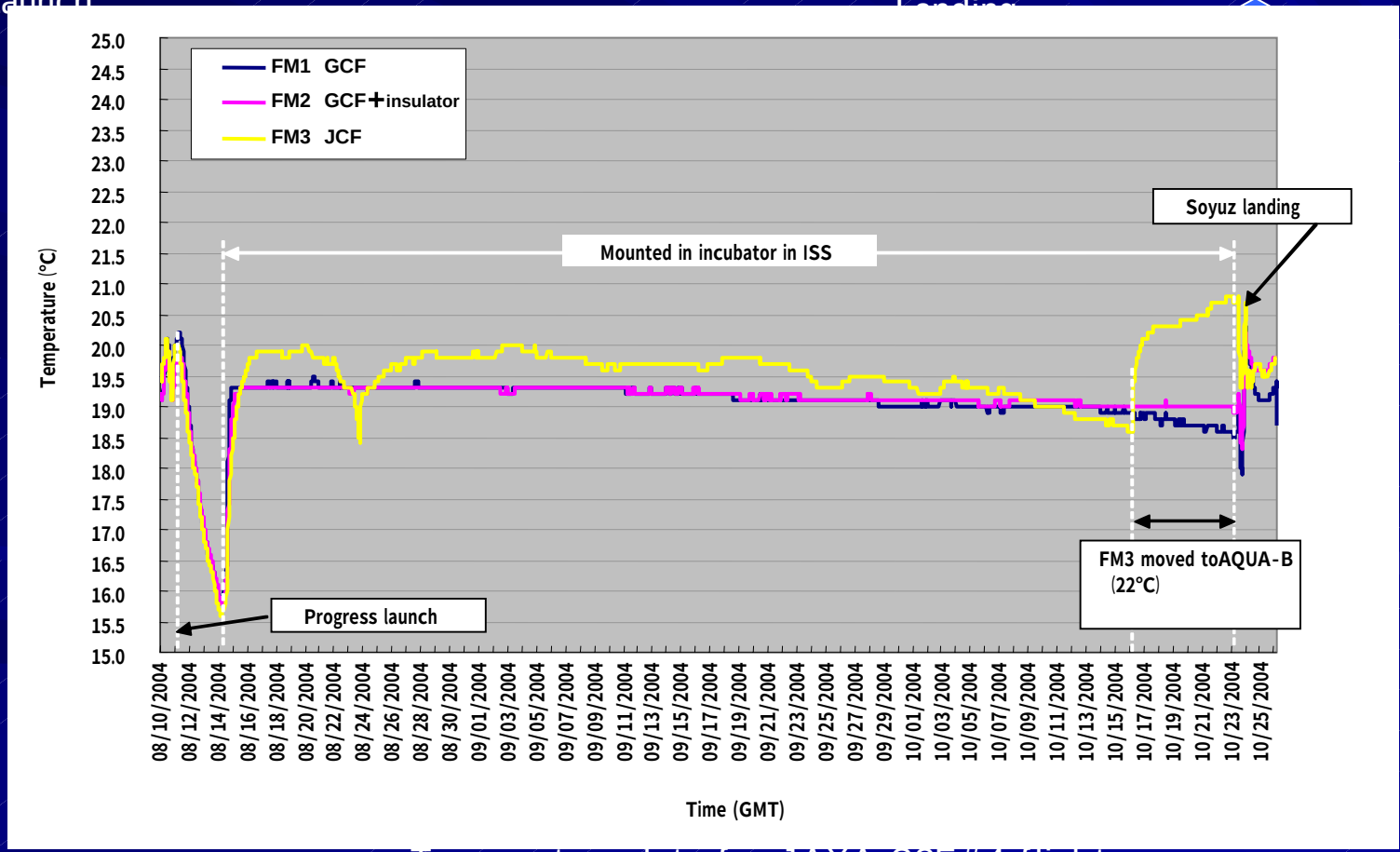
- 37 GCBs (38 proteins) were launched.
  - Users: 32 proteins
  - JAXA: 6 protein (alpha-Amylase, Lysozyme)
- Single crystals which seemed to diffract well were obtained in 22 proteins.
  - 21 single crystals were obtained out of 27 samples which were successfully optimized crystallization condition.
- Temperature in ISS and on the way back from ISS was stable.
- No leakage from capillaries was observed.
- PEG 64 conditions / 74 conditions (86%)
- Technical supports of harvesting and cryoprotecting crystals successfully contributed to X-ray diffraction experiment.
- Thermos bottle was effective, but same as GCF with alkan.
- Correlation between GCB and JCB was reasonable, however there are still some points to be improved.
  - Leakage
  - Crack
  - Structural improvement



Launch

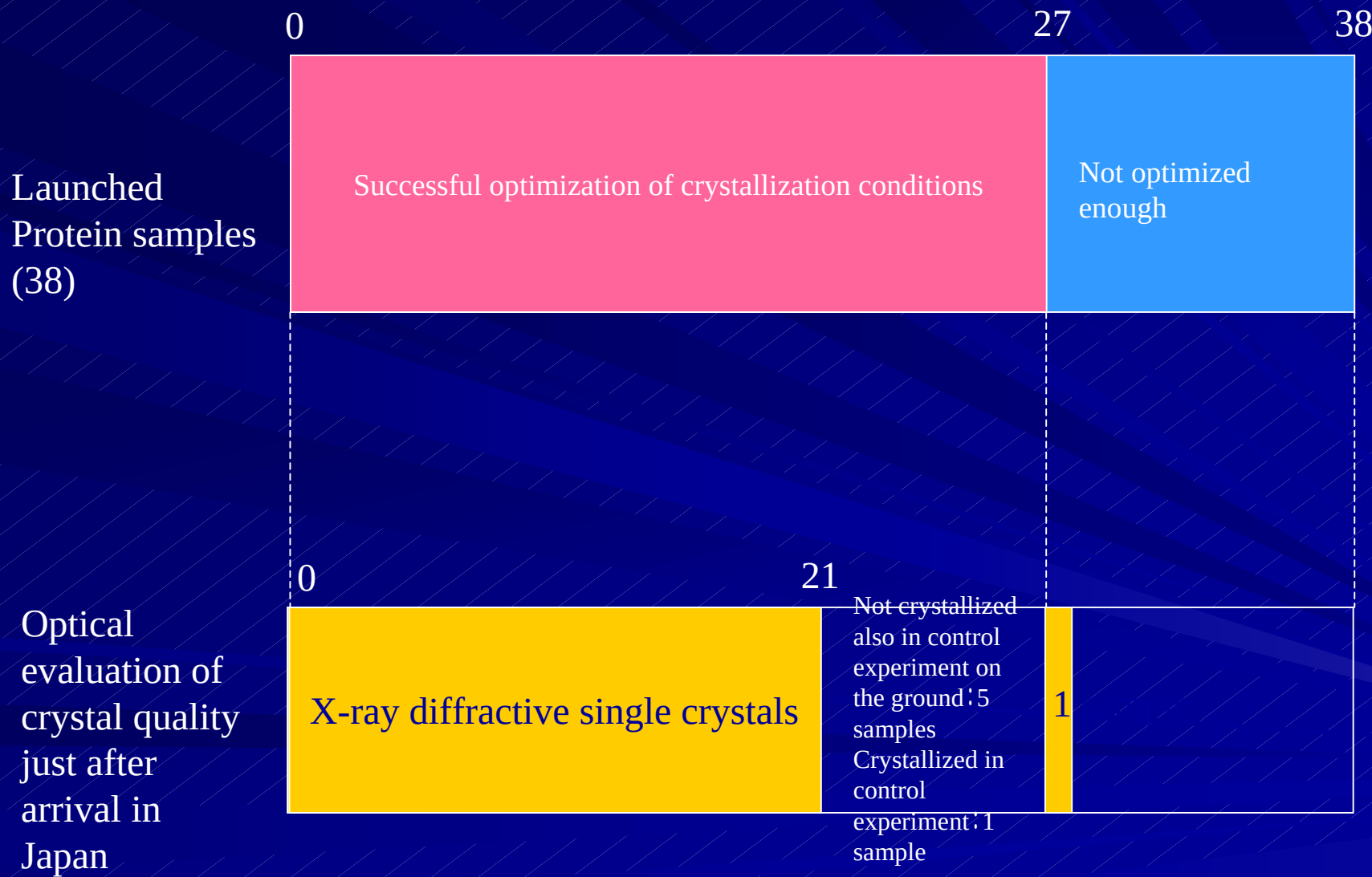


Landing

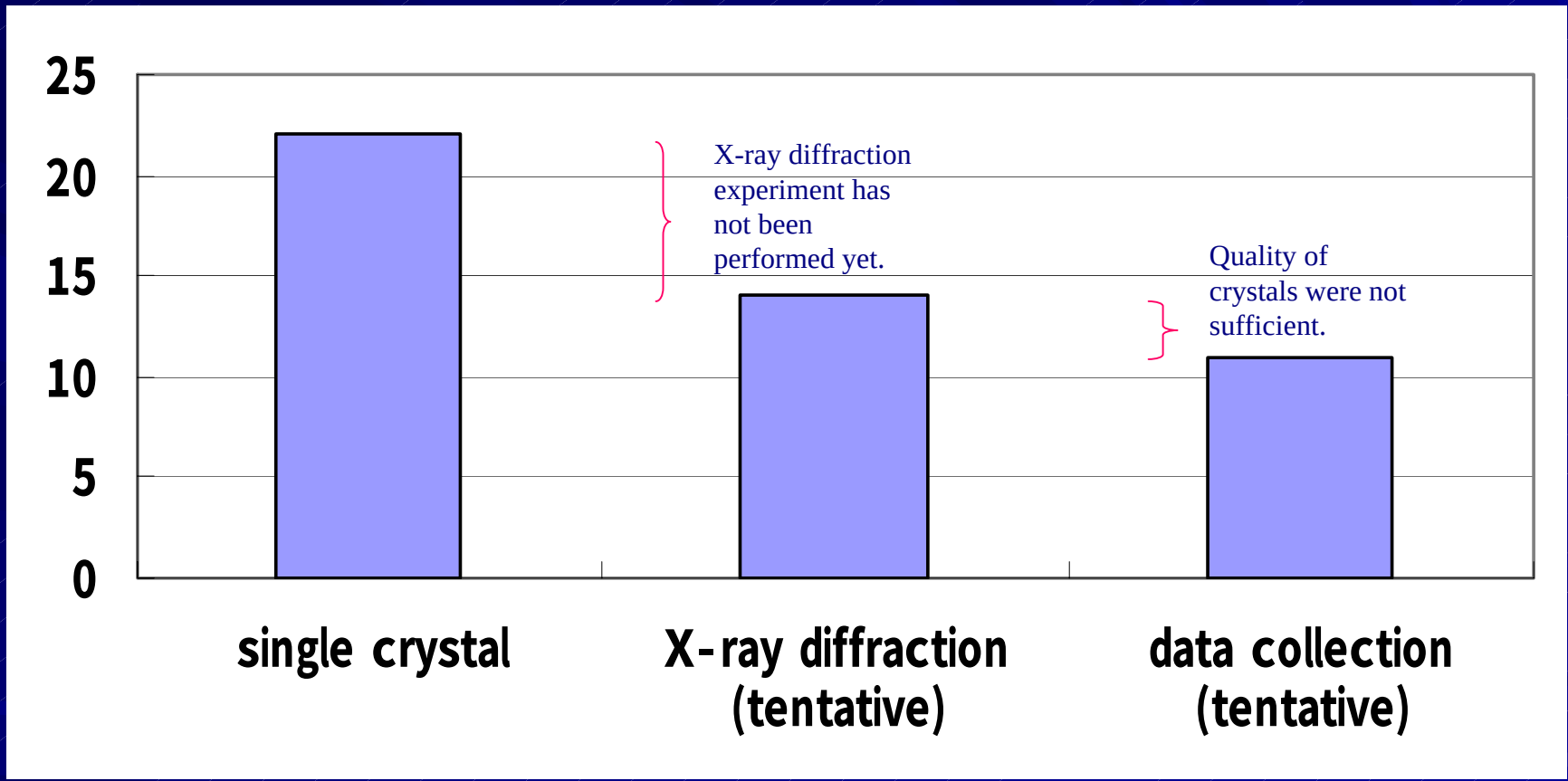


Temperature data for JAXA-GCF#4 flight

Tentative results of JAXA-GCF#4



From obtaining single crystals to diffraction data collection



2004.12.

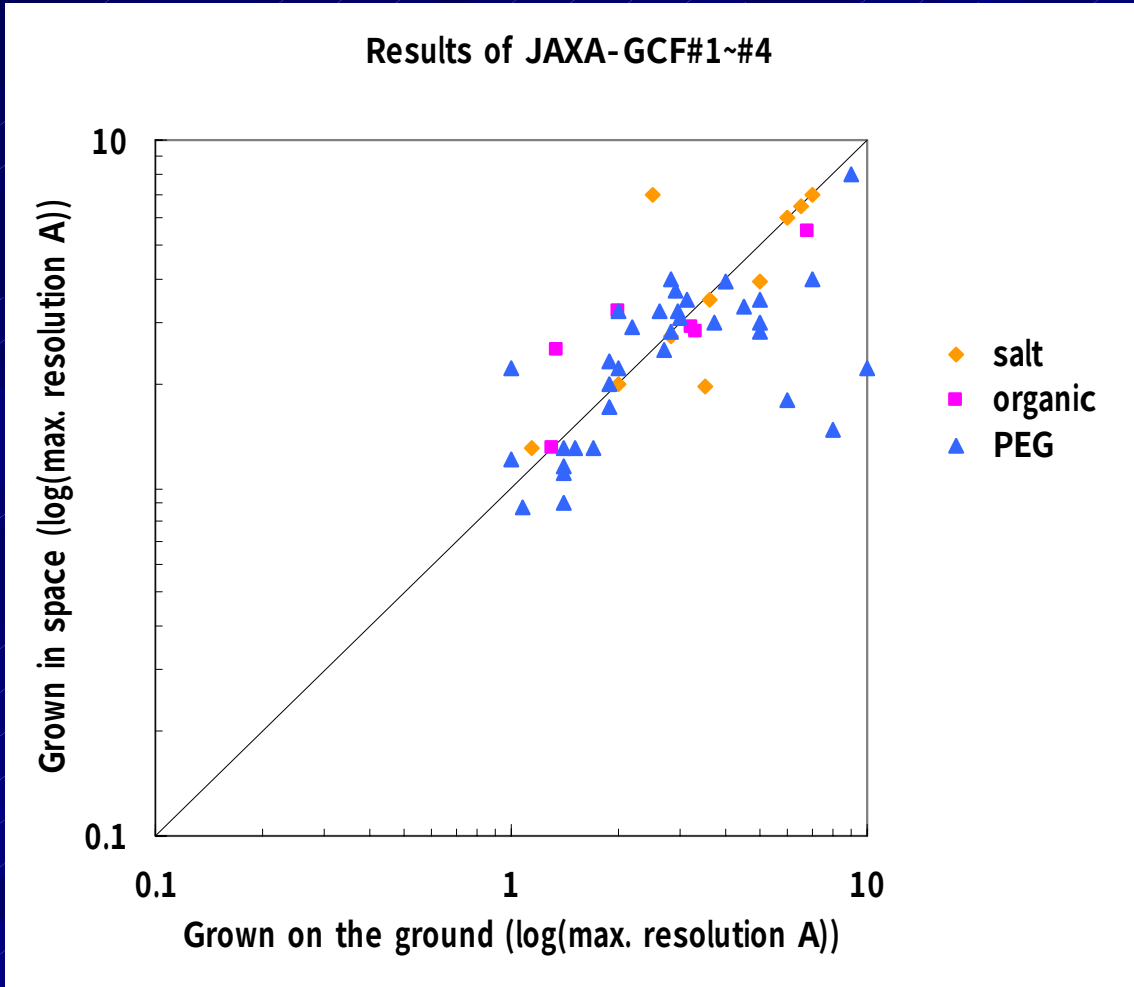
Tentative results of diffraction data

These samples will be further examined.

	ground experiment (A)	space experiment (A)	precipitant
1	2.5	2.2	PEG
2	6.8	5~6	Organic
3	3.11	3.2	PEG
4	2.6	2.8	Salt added with PEG
5	3.2	1.8	PEG
6	2.33	1.46	PEG
7	3.2~4.0	3.0~3.2	Salt + PEG
8	18	8	PEG
9	2.6	2.2	PEG
10	3.4	2.0	PEG
11	2.3	1.8	PEG
12	-	3.0	PEG
13	1.9	2.0~2.4	Salt added with PEG
14	1.08	0.93	Salt added with PEG
15	3.5	1.85~2.05	Salt

9 more samples (8 proteins) will be performed X-ray diffraction experiment in the near future

Comparison between Maximum Resolution of Crystals grown on the ground and in space



Data are collected from crystals grown in JAXA(NASDA)-GCF#1, #2, #3 and #4 (tentative) space experiments

まとめ

- 有用な回折データが取得できる確率の大幅向上
  - 過去のトラブルの改良
  - 取り出し、凍結のサポート
- 結晶化条件の絞込みにはまだ課題あり
- 高密度容器は改良中