STS-107 JUSPRO Experiments

1. Business and crystallography
2. Outline of crystallography
3. JUSPRO experiment

Protein crystallography and business

Resources
Human and animal cells
Plant and bacterial cells

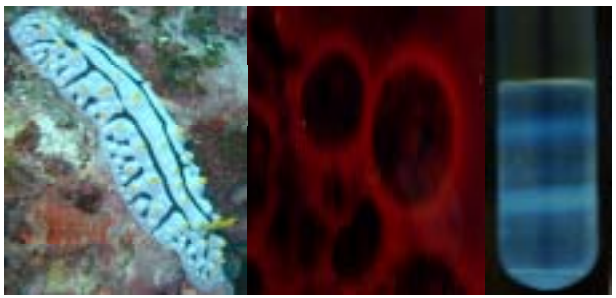
Genome
A set of chromosomes
Cluster of genes

DNA
Gene
ATGC

mRNA
Messenger of genetic information

Protein
Polymer of amino acid subject to mRNA

Individual
Metabolism
disease



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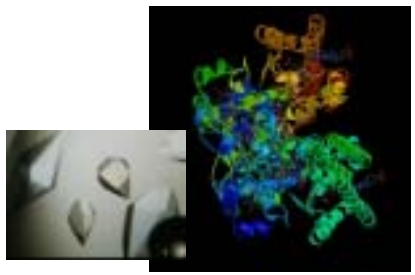


Genomics

Function

Complete of human genomics

Current focus Functions of Genes (proteins)

Patent: Structural information with function

method
- Bioinformatics
  protein 3D
- DNA micro array
- Transgenic organism
- Genetic analysis

Gene expression (bioprocess) business

Outlines for Protein Crystallography

Crystal

Cryo and diffract

Diffraction intensities

Phase information

Electron density map

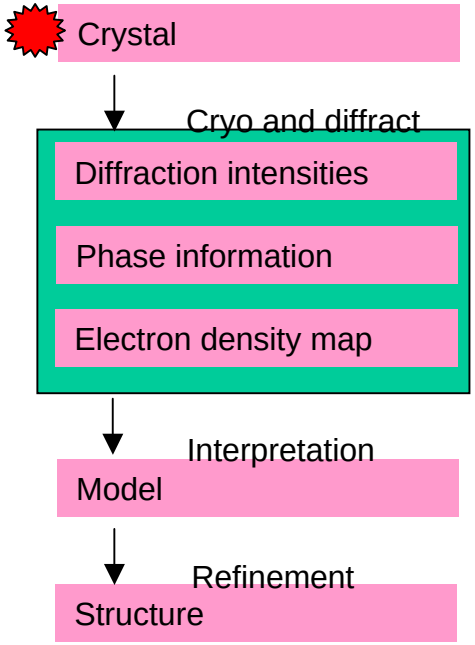
Interpretation

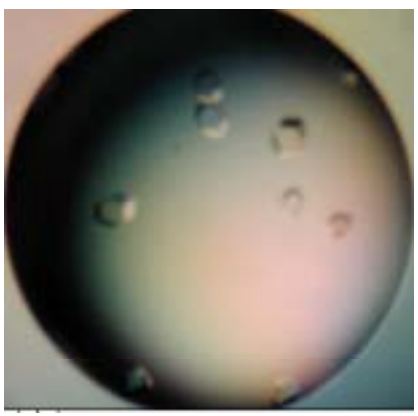
Model

Refinement

Structure

Crystallization



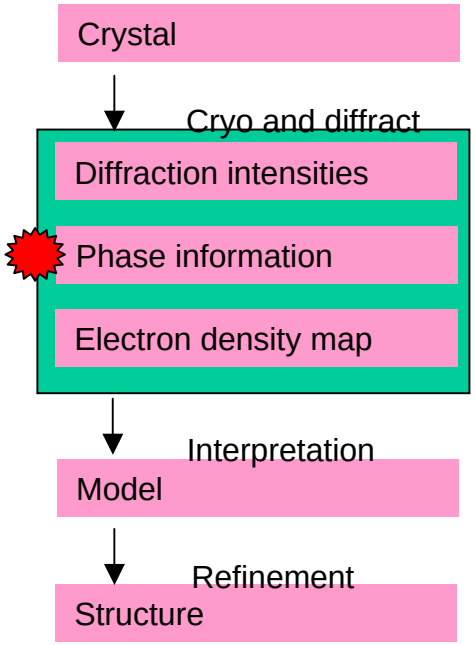


Required items:
Protein (mono spread, 99% pure, 10 mg)
Precipitant (*e.g.* AS, PEG)
Additive (*e.g.* Zn, Ca, MPD)
Detergent

To be observed:
3D crystal with shape edge, polarization

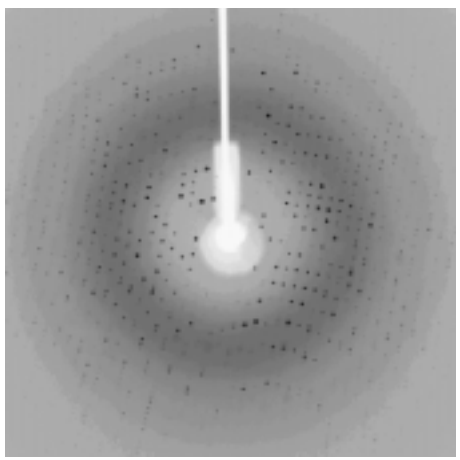
To be obtained:
Diffracting crystal
with small mosaic, high resolution

Diffraction experiment



Required items:
Crystal (freeze; native, derivatives)
X-ray (monochromatic, white)
Detector (imaging plate, CCD)

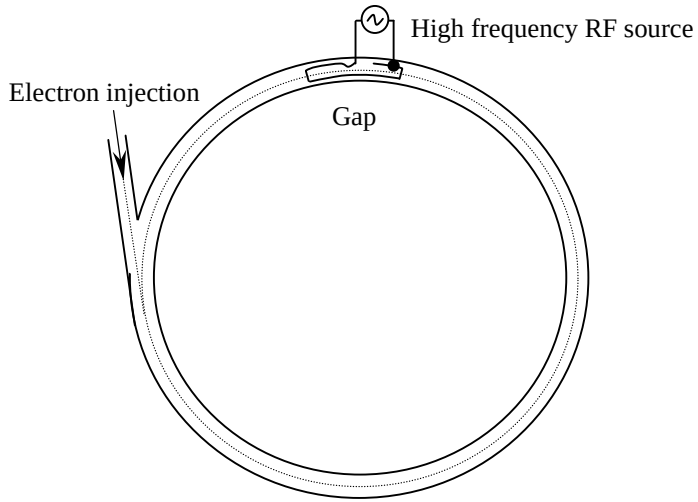
To be observed:
Diffraction intensity distribution



To be obtained:
Phase information (structure factor)
with index, intensities, error, phase angle
by SIR, MIR, MIR-AS, SAD, MAD

Diffraction experiment: Synchrotron

High-energy electron emits white ray called synchrotron radiation as it traverses in magnetic field.



X-ray specificity:

High flux density

strong, brilliant: 20 μm small crystal

Tunable

flexible & precise wavelength: anomalous dispersion

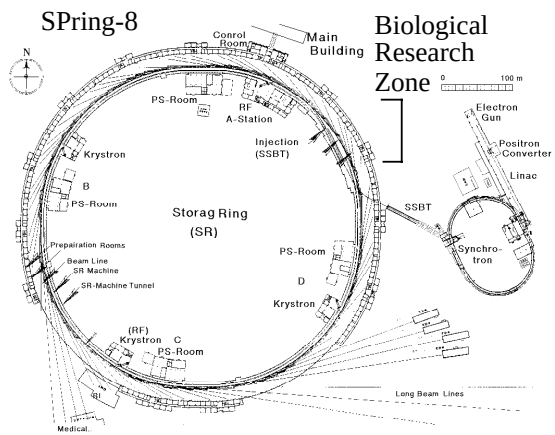
Low diversity

parallel: large unit cell

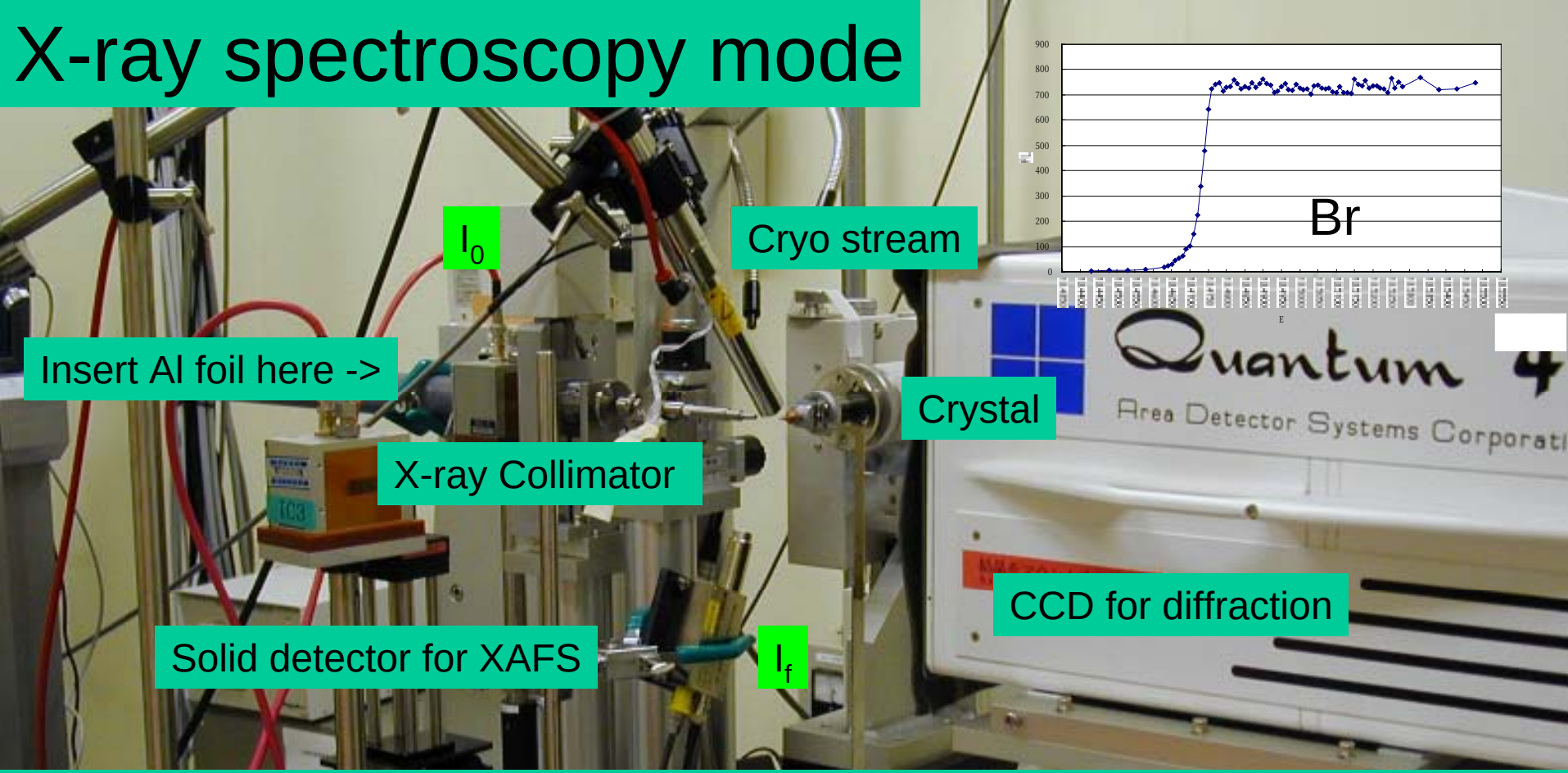
Application on MAD, SAD:

Florescence spectrum

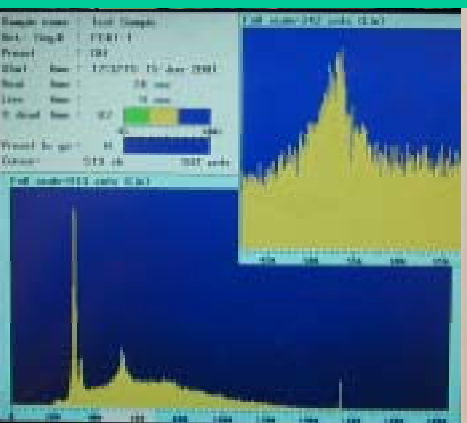
multiple wave length data collection



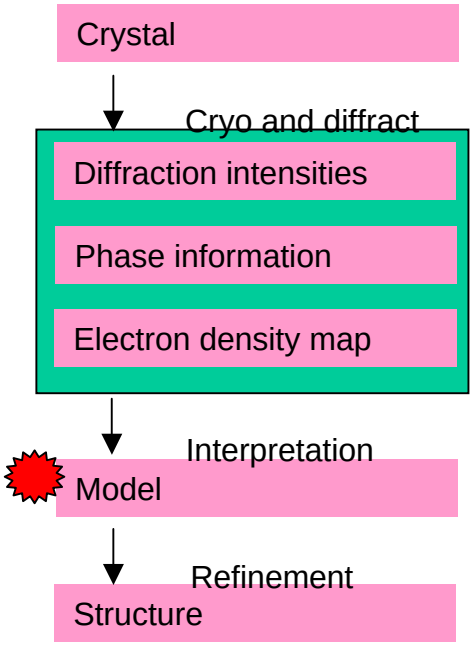
X-ray spectroscopy mode



Multi Channel Analyzer (window) -> Digitized signal from SCA -> XAFS (FL) Wizard



Modeling and Refinement



Required items:

Electron density map

Amino acid sequence

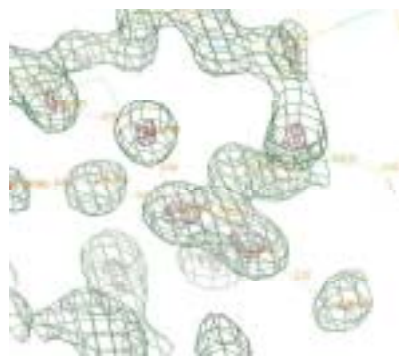
Computer graphics and computation

To be observed:

Fit of model and E.D. map

To be obtained:

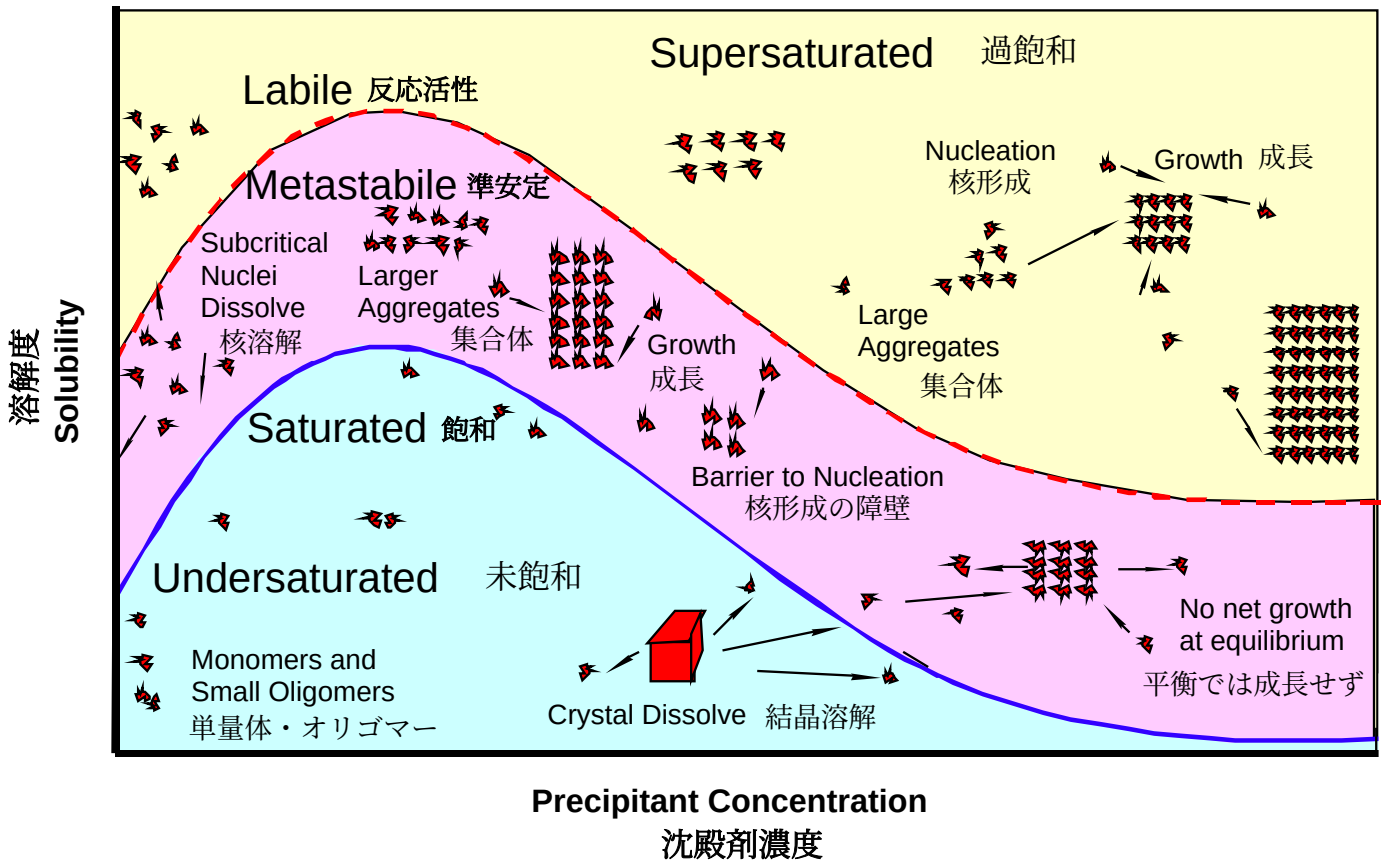
Atomic structure model



Crystallization

The phase diagram and the physical events

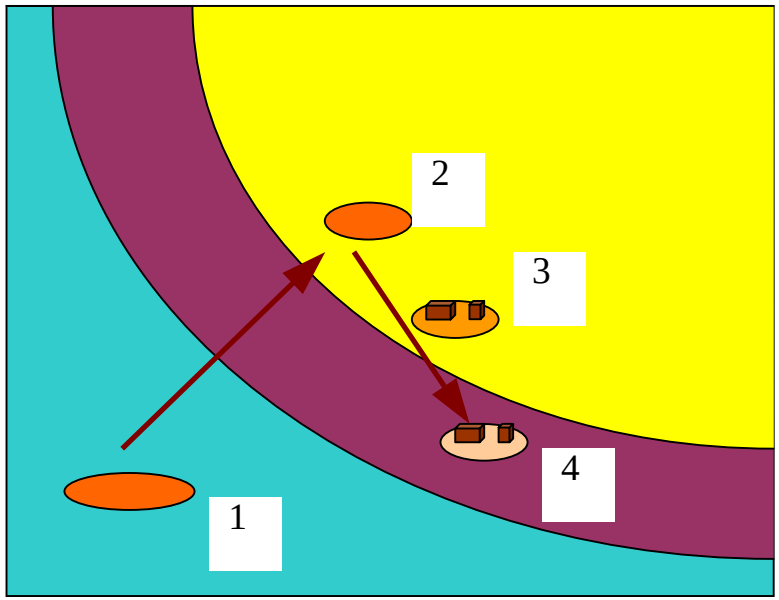
相図と結晶化に関する現象



After Figure 4.5. The phase diagram. Alexander McPherson, *Crystallization of Biological Macromolecules,* p133.Cold Spring Harbor Laboratory Press, 1999.

Crystallization

Hanging drop technique
ハンギングドロップ蒸気拡散法によるタンパク質結晶化



Protein concentration

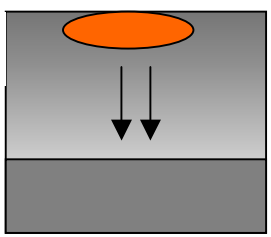
タンパク質溶解度

Supersolubility curve
過飽和度曲線

Solubility curve
溶解度曲線

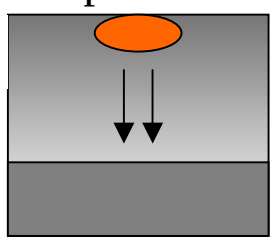
Precipitant concentration 沈殿剤濃度

1 蒸発



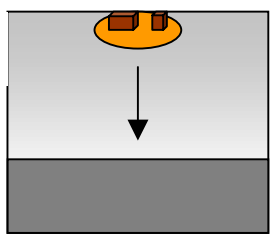
Evaporation of water increases the concentration of precipitant and protein at the same rate.

2 濃縮



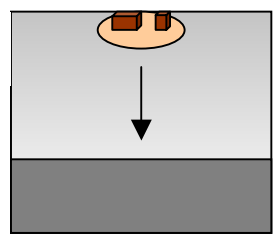
Therefore, the value of supersaturation in the drop increases.

3 核形成



As a result protein precipitates hopefully as crystals.

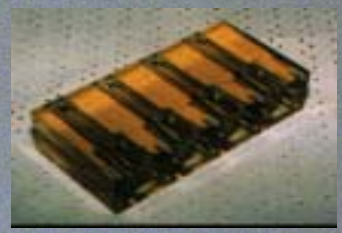
4 成長

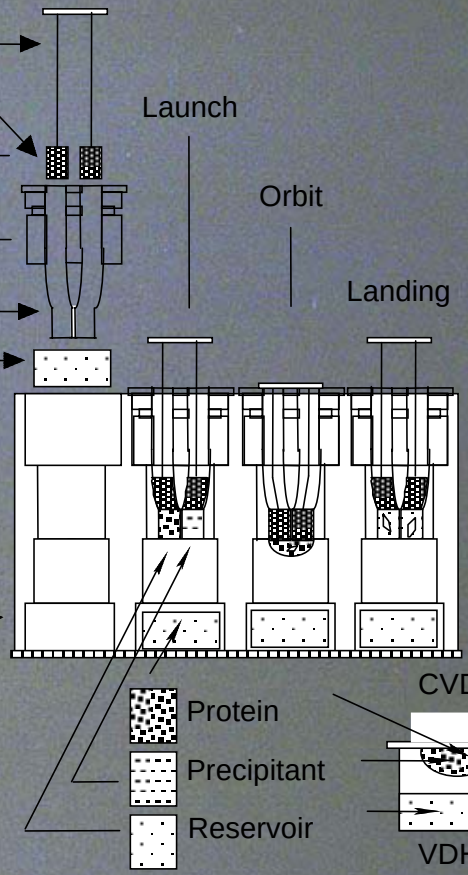


And the concentration of protein decreases

Crystallization under microgravity

Control of Flow (Convection)



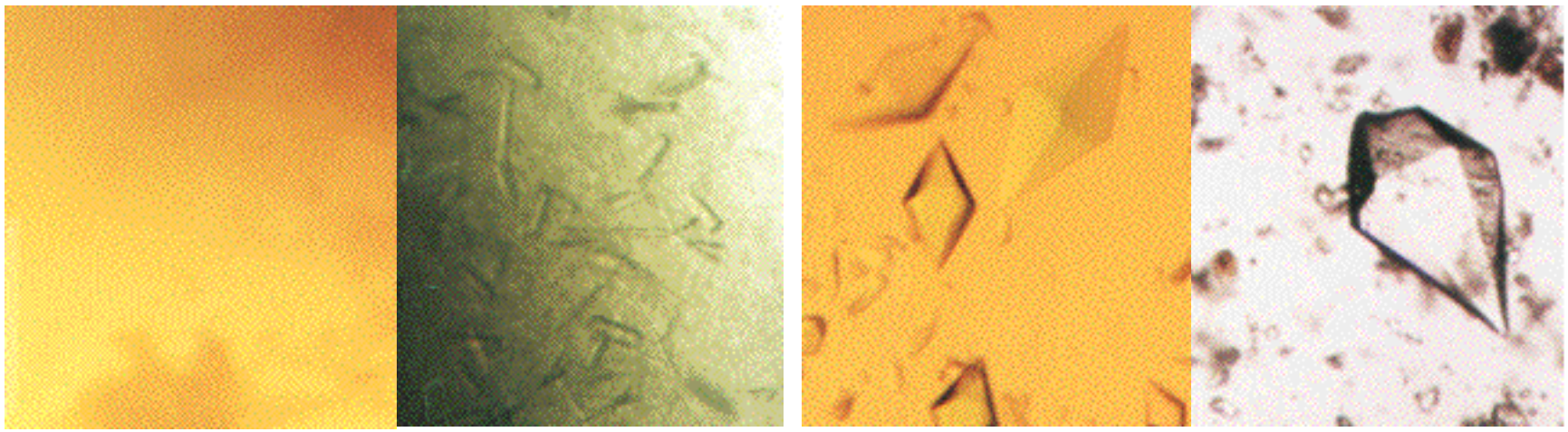


NASA

Crystallization under microgravity

Adrenodoxin reductase (ADR) Isopropylmalate dehydrogenase (IMD)

Ground control Microgravity Ground control Microgravity



1 mm

Table . Items for post flight analysis and results.

		ADR		IMD	
No. and Item	Method / Item	Ground	Space	Ground	Space
1. Presence of crystals	Polarized microscope	No	Yes	Yes	Yes
2. Space group or system	SR X-ray diffraction	NA	*monoclinic	$P3_221$	$P3_221$
3. Specific gravity	Linear gradient	NA	1.25	1.21	1.21
4. Molecular structure	PX resolution / R-factor	NA	2.8Å / 0.20	2.3Å / 0.19	1.9Å / 0.20
5. Residual protein conc.	Dye binding	7 mg/ml	8 mg/ml	4 mg/ml	3 mg/ml

*, to be confirm; NA, Not applicable.

Crystallization trials on adrenodoxin reductase and 3-isopropylmalate dehydrogenase in STS-84 NASADA/UAB

Ground-Space crystallization condition shift tabling

Crystallization conditions are different between 1 G and microgravity.

It is caused by the difference of:

a. Crystallization bin (drop, reservoir, vapor space)

b. Temperature (4, 10, 15, 20, 37^{o}C)

c. Mixing and start of growth

(suspended nucleation, resumed growth)

So on

These differences are obscure in comparative study

Comparative studies
between
Ground experiment result diagram
and
Space experiments result diagram
gives
Ground-Space crystallization condition shift tabling

Difference in protein crystallization can be classified as a different precipitant condition and additive.

JUSPRO experiments
Tabling (glucose isomerase)
new crystallization (guanyl cyclase)