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A visit to the Structural Biology Laboratory

at Marshall Space Flight Center

Microgravity Crystallization

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for Structural Biology

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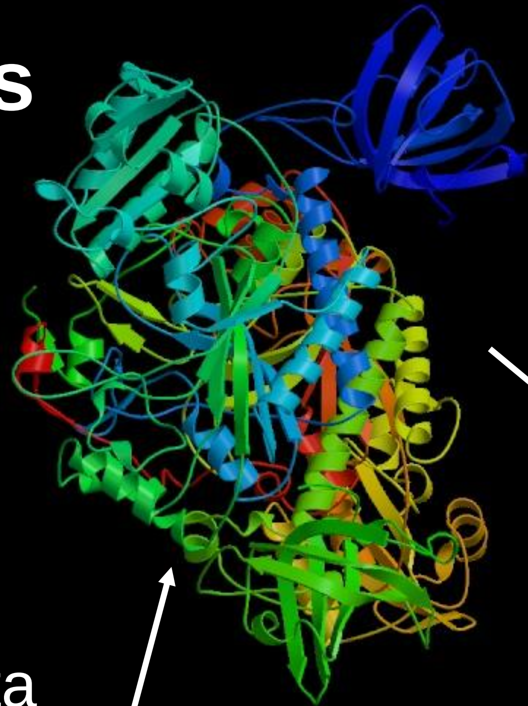
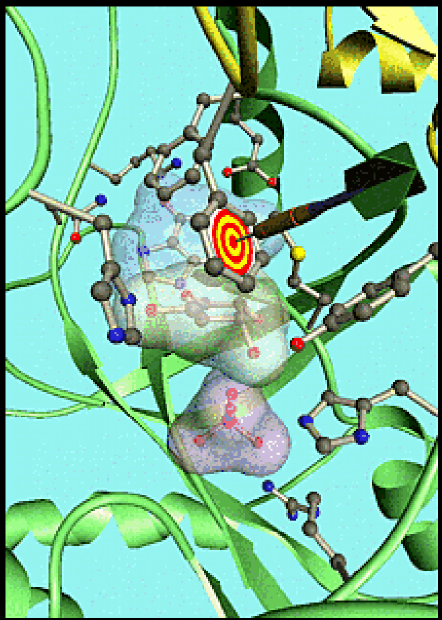
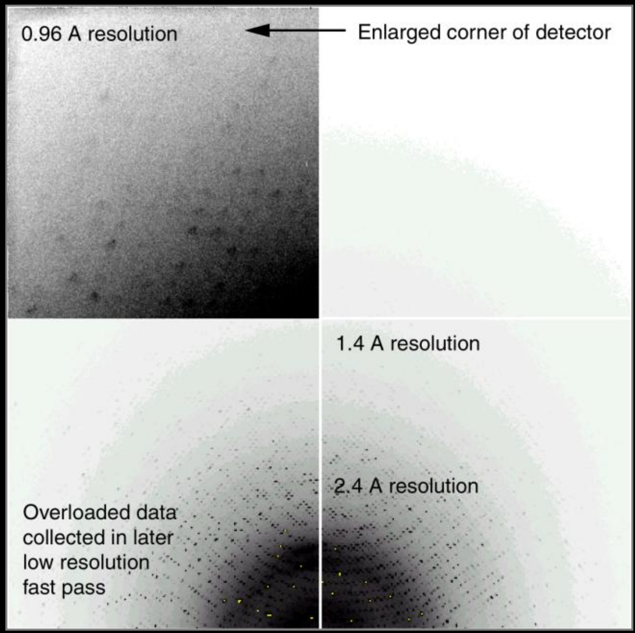
Crystallography process

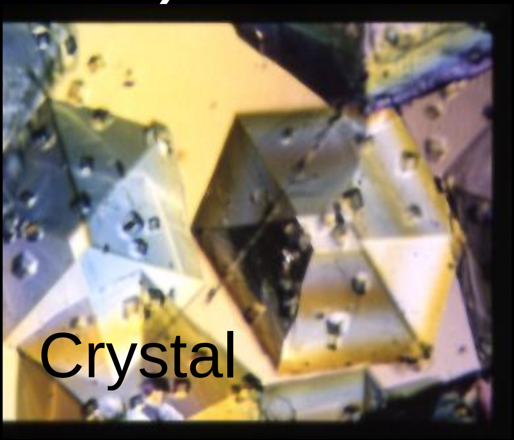


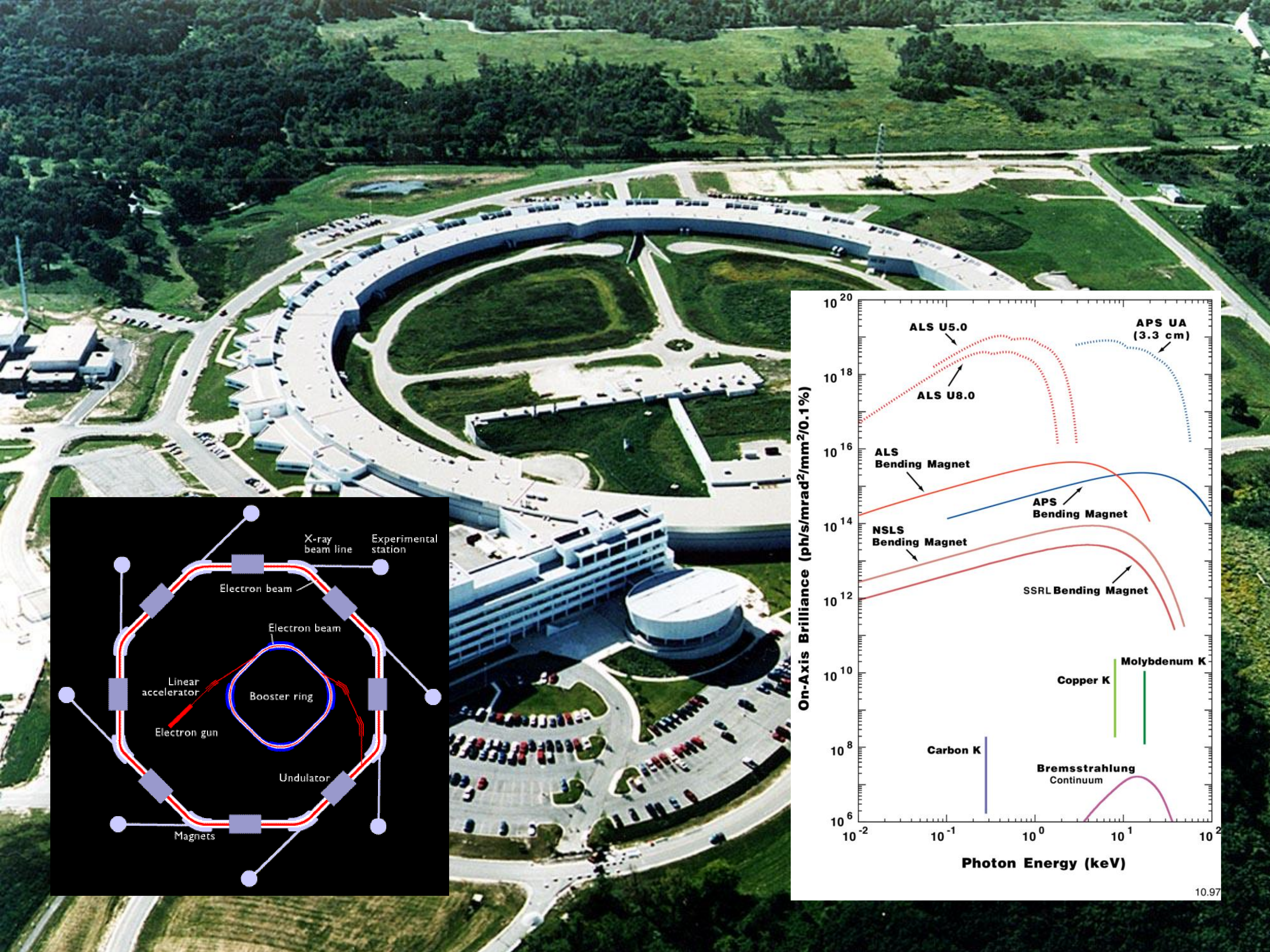
Target
for drug



X-ray data







X-ray beam line
Experimental station
Electron beam
Electron beam
Linear accelerator
Booster ring
Electron gun
Undulator
Magnets
ALS U5.0
APS UA (3.3 cm)
ALS U8.0
ALS Bending Magnet
APS Bending Magnet
NSLS Bending Magnet
SSRL Bending Magnet
Molybdenum K
Copper K
Carbon K
Bremsstrahlung Continuum
On-Axis Brilliance (ph/s/mrad²/mm²/0.1%)
10^{20}
10^{18}
10^{16}
10^{14}
10^{12}
10^{10}
10^{8}
10^{6}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
Photon Energy (keV)
10.97

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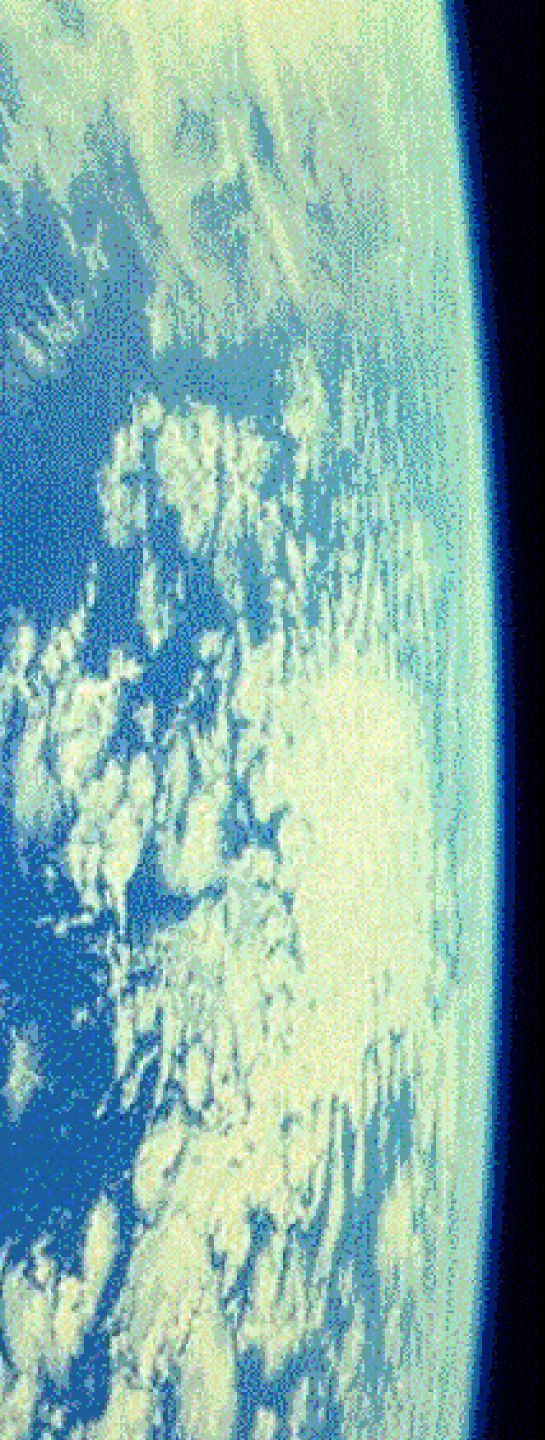
Order in a crystal

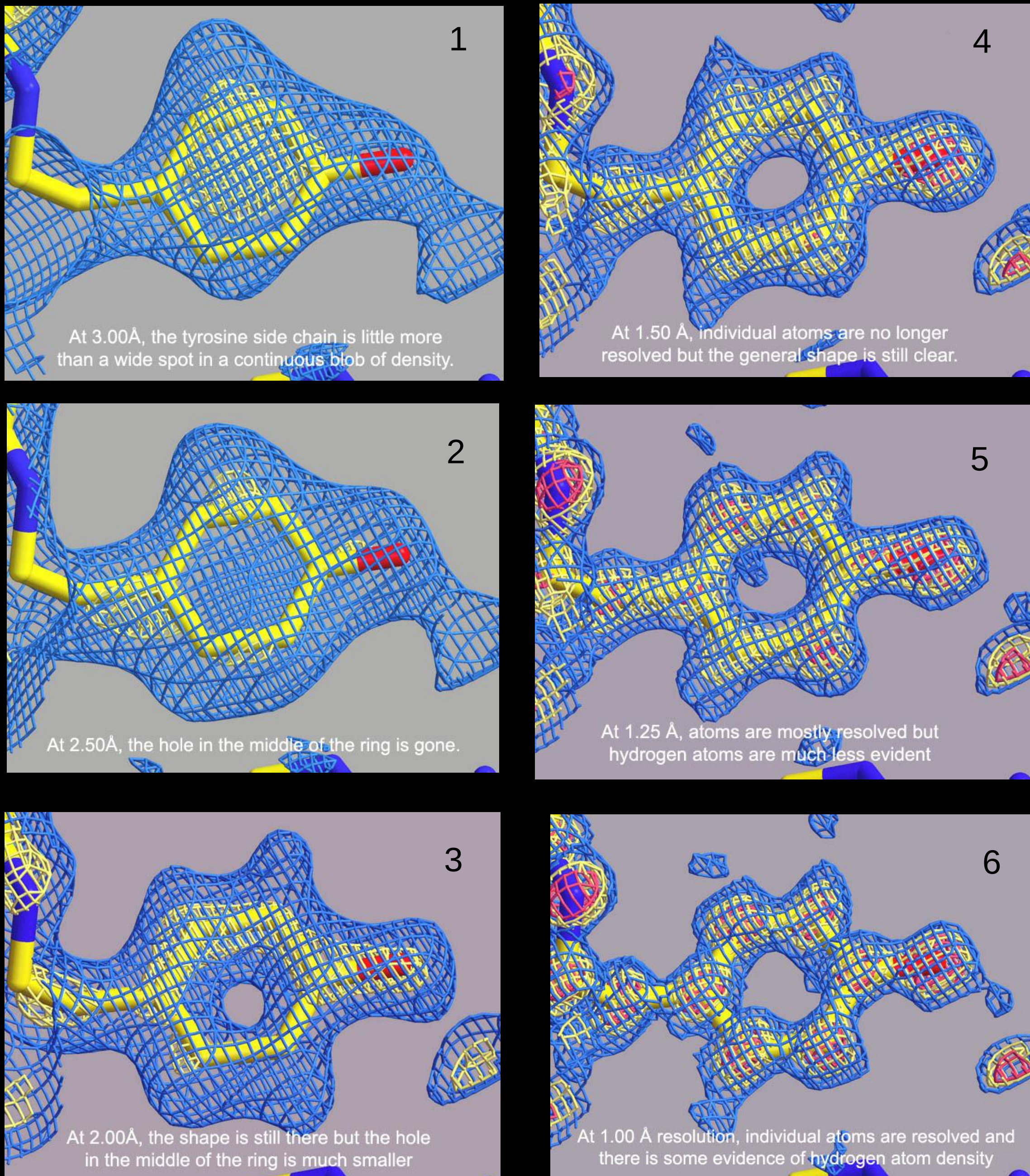
- An atom only contributes to the intensity of the diffraction spot if its disorder relative to symmetry-related atoms is small compared to the resolution of the spot.
- The internal order in a crystal can be characterized by a correlation length, *i.e.* the distance over which the two atoms in a unit cell are accurately related by the symmetry operators.
- For random disorder, as resolution increases, the effective correlation length decreases and the number of unit cells contributing coherently to the diffraction decreases.
- Random disorder is a major contributor to the reduction in diffracted intensity with increasing resolution (this is why temperature factor has been renamed atomic displacement factor).



Short and long-range order

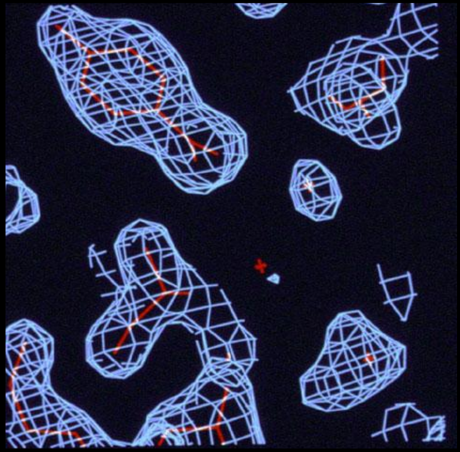
- The order can be described as short-range or long-range.
- Short-range disorder gives rise to global effects in reciprocal space (random disorder reduces resolution). Short range order is determined with structural data collection.
- Long-range disorder gives rise to localized effects in reciprocal space (mosaicity broadens spots).
- Long-range disorder amenable to investigation by reflection analysis to reveal mosaicity and domain structure effects.

Quality (Resolution)

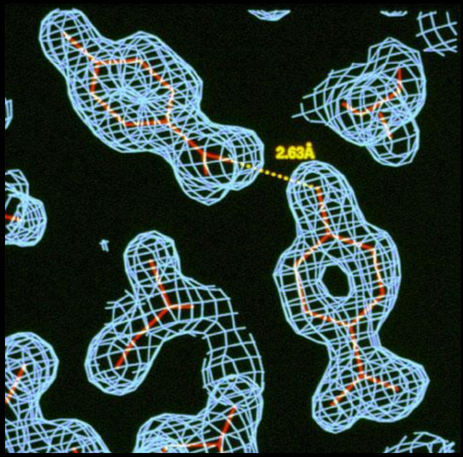


Sean Parkin. UK

Low resolution ground



High resolution microgravity

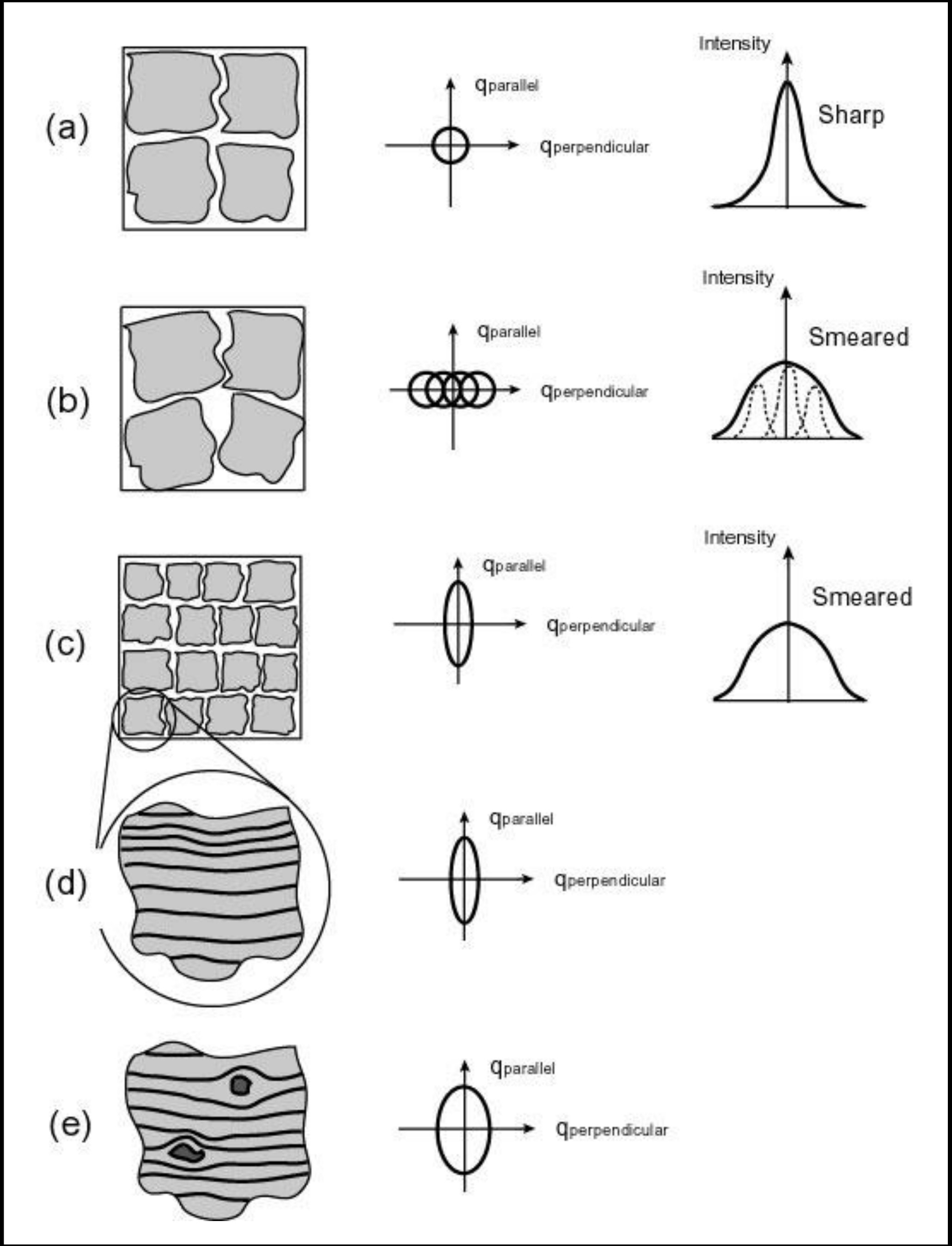


Long range order

Darwin proposed the mosaic model of crystals consisting (a) of perfectly ordered volumes (domains) slightly misaligned with each other.

In addition to having (b) small random misalignments the domain can be of (c) varying volume and the unit cells in the crystal (d) can vary due to mutations *etc*.

Misalignment, volume and unit cell variation all have effects in reciprocal space (smearing out reflections) which can be represented by the Darwin model.



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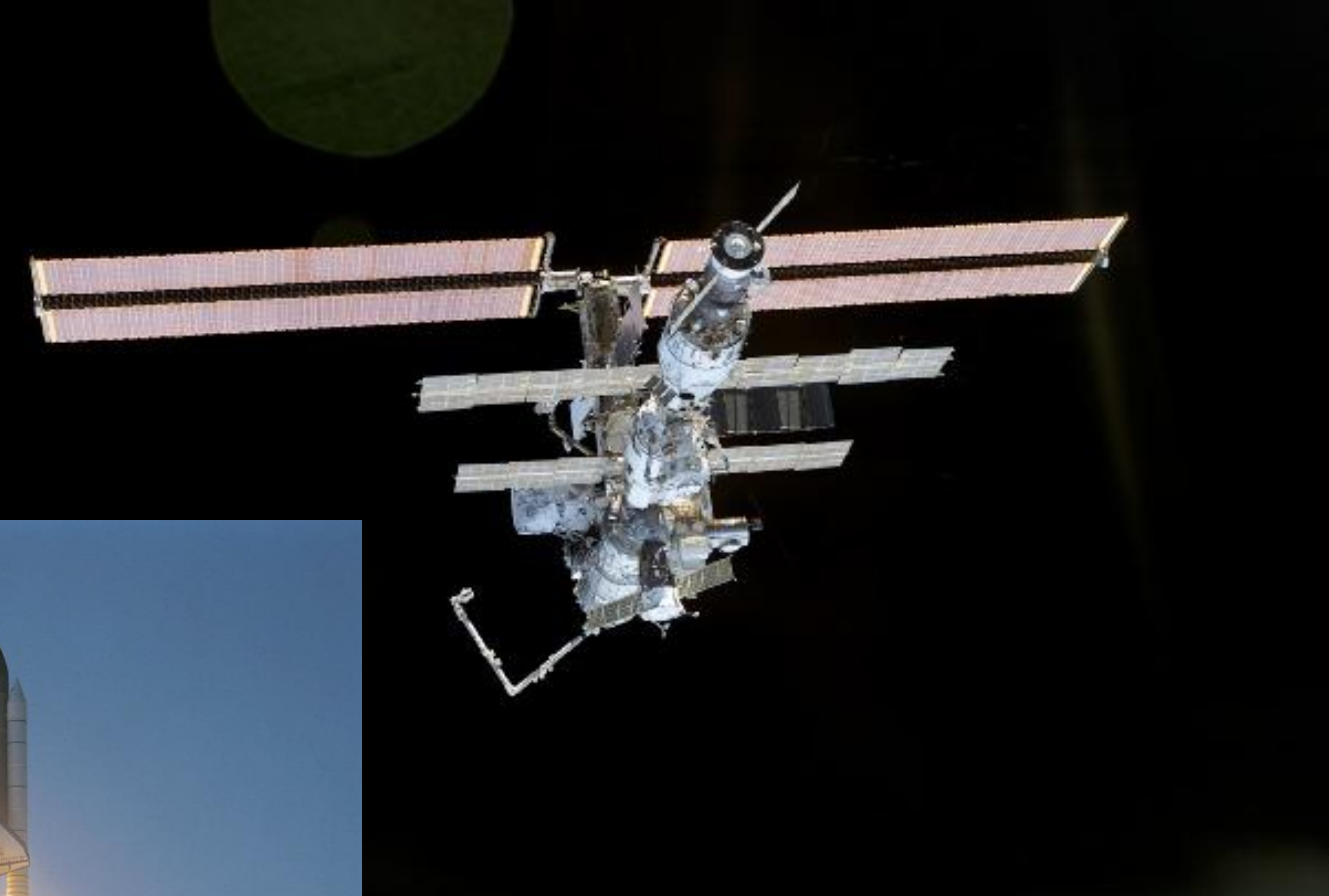
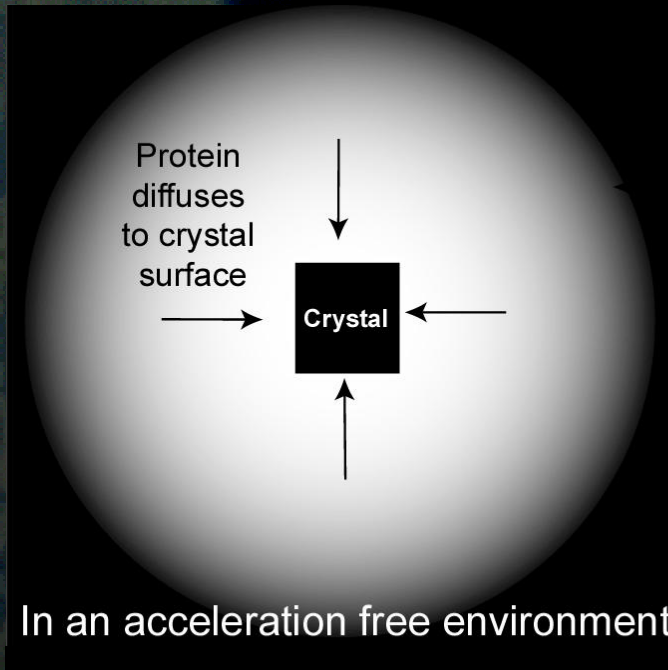
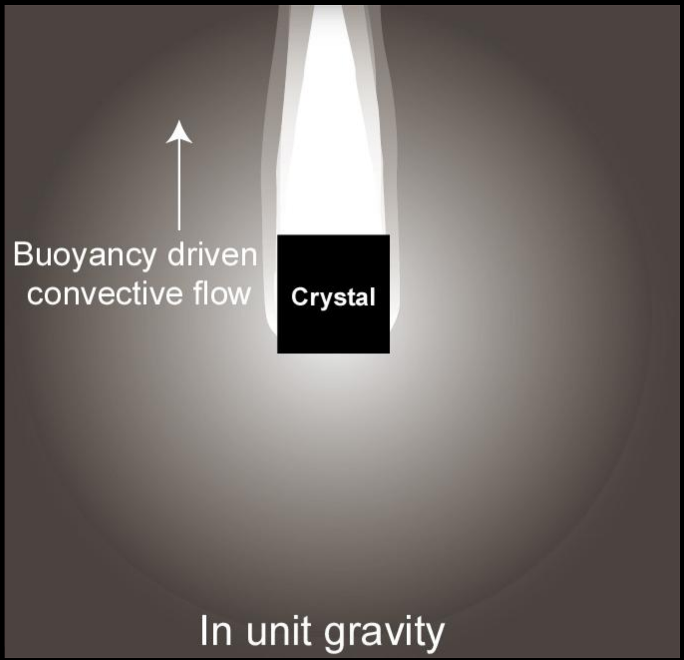
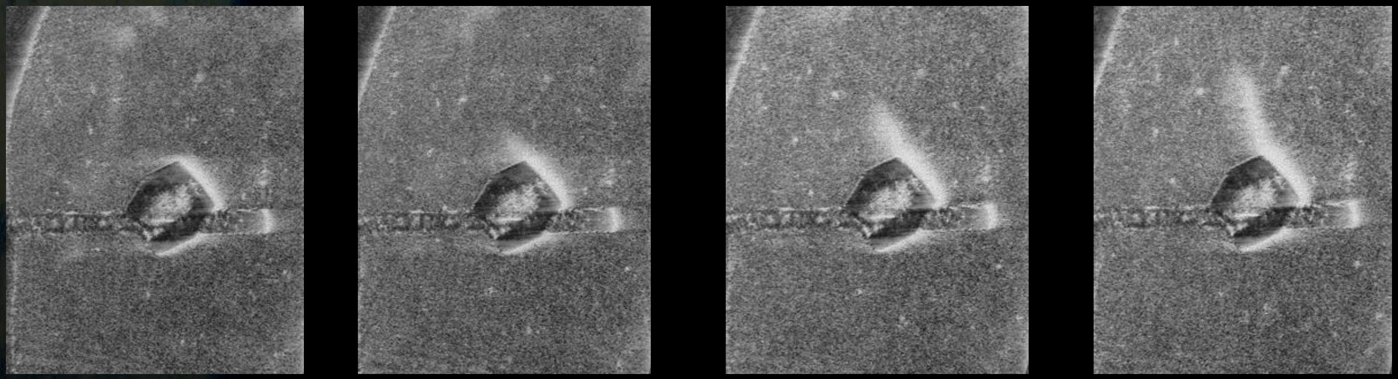


So why is NASA interested?

Growing crystals in microgravity







In microgravity:

Buoyancy driven convection effects are greatly reduced:

- A zone of depleted material is formed around the crystal.
- Crystal growth is dominated by diffusion transport.

Sedimentation of the crystals is greatly reduced:

- Crystals are suspended in nutrient for a longer time.

Experiments are small volume, have low mass and can have high scientific and commercial return:

- Many experiments can be flown at one time and even a low success rate would still result in

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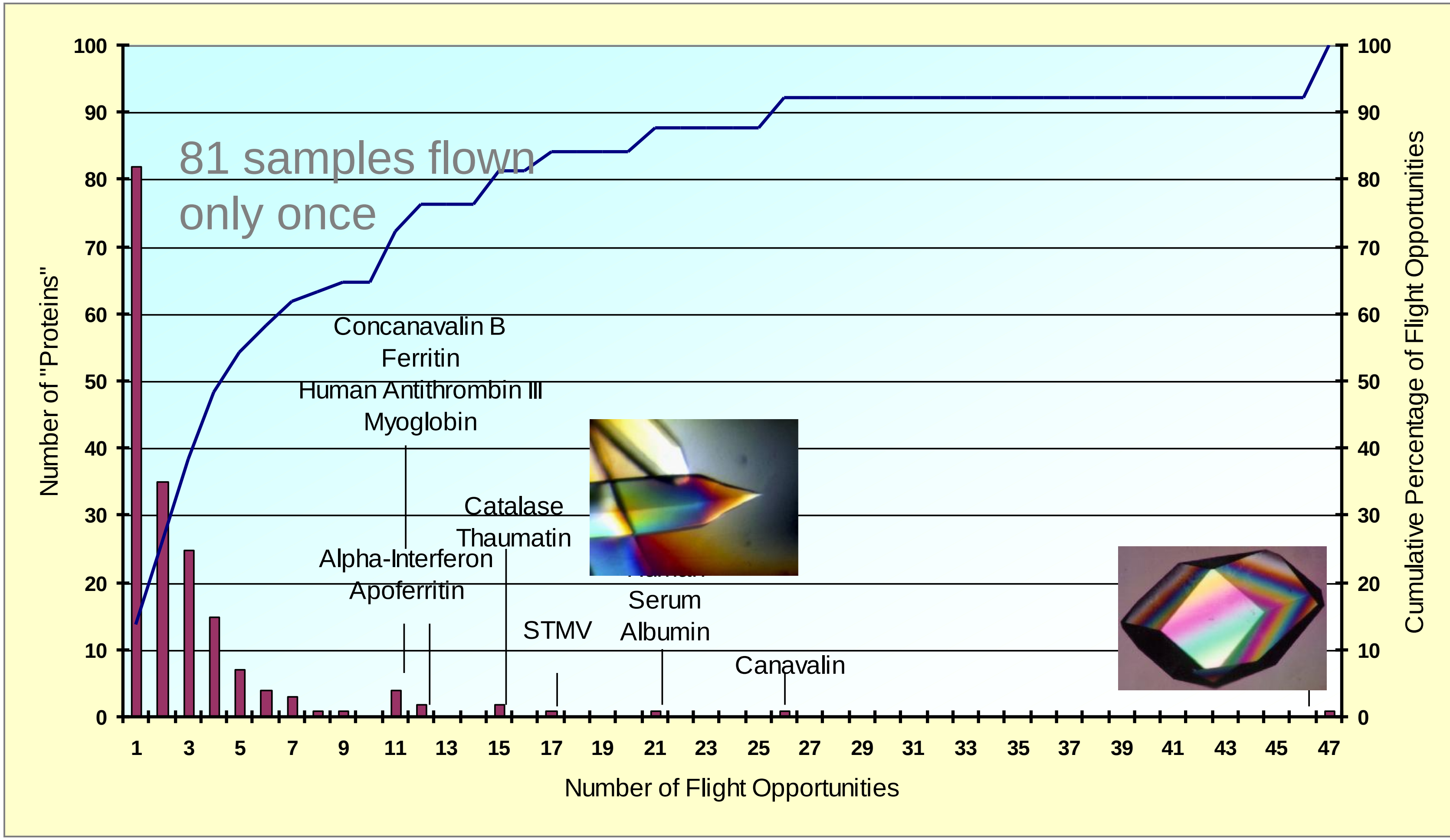
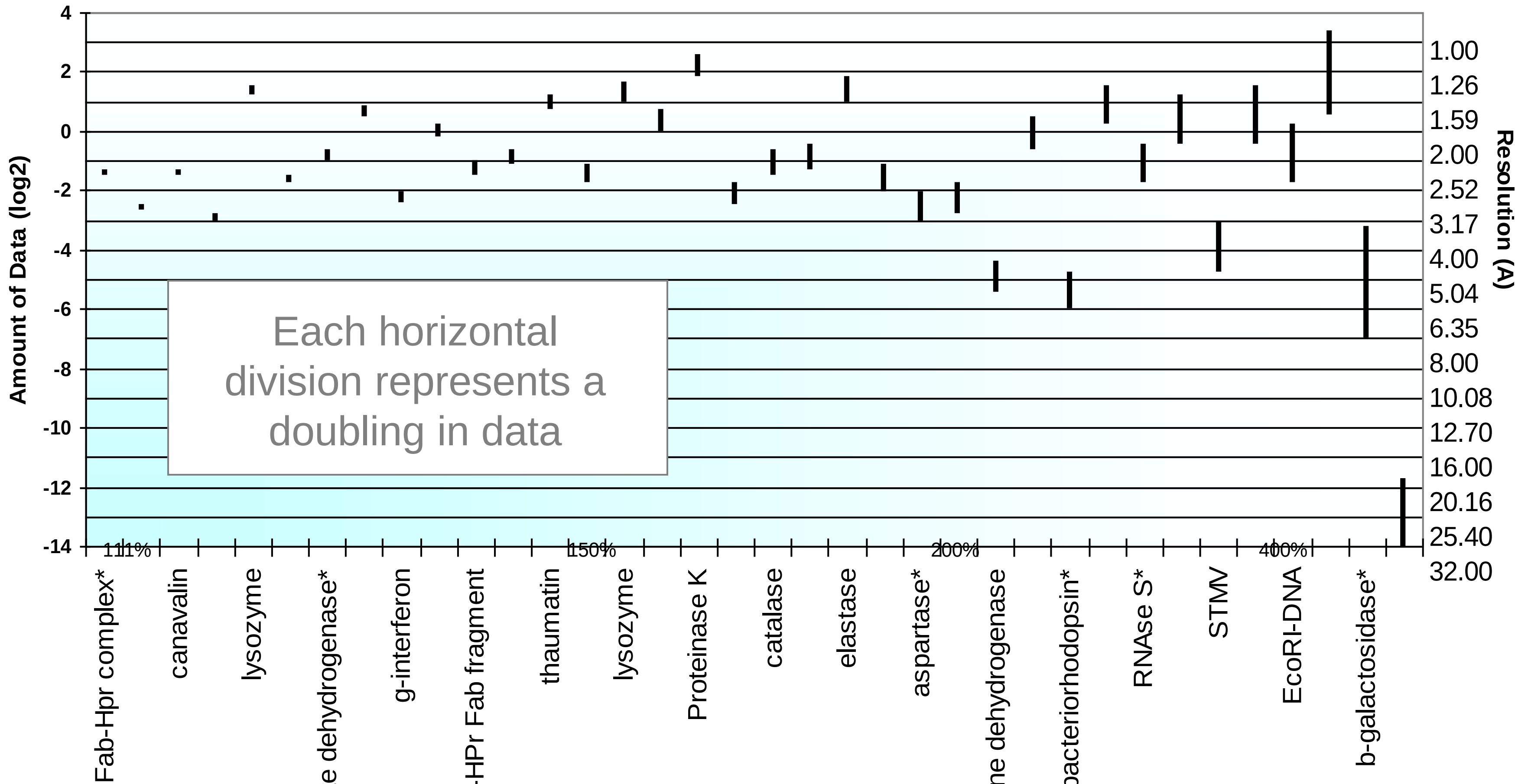
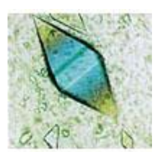
Over 185 different macromolecular samples have flown. Some are frequent flyers, others have flown only a limited number of times

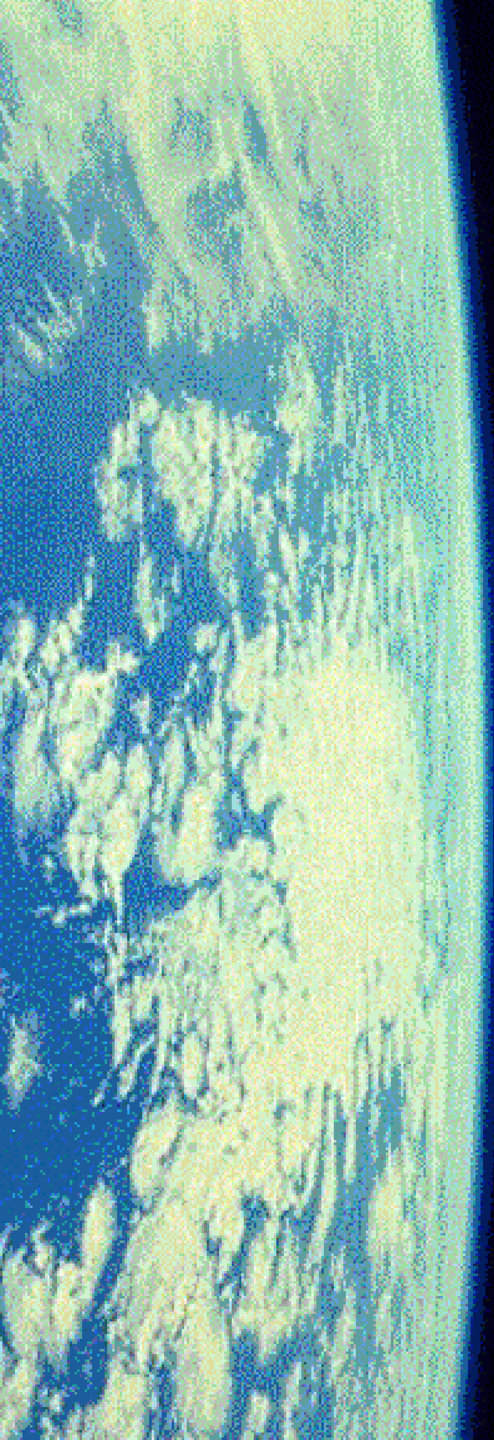


Microgravity and Macromolecualr Crystallography: Kundrot, C.E., Judge, R.A., Pusey, M.L., & Snell, E.H. Crystal Growth and Design. Crystal Growth and Design, 1, 87-99 (2001).

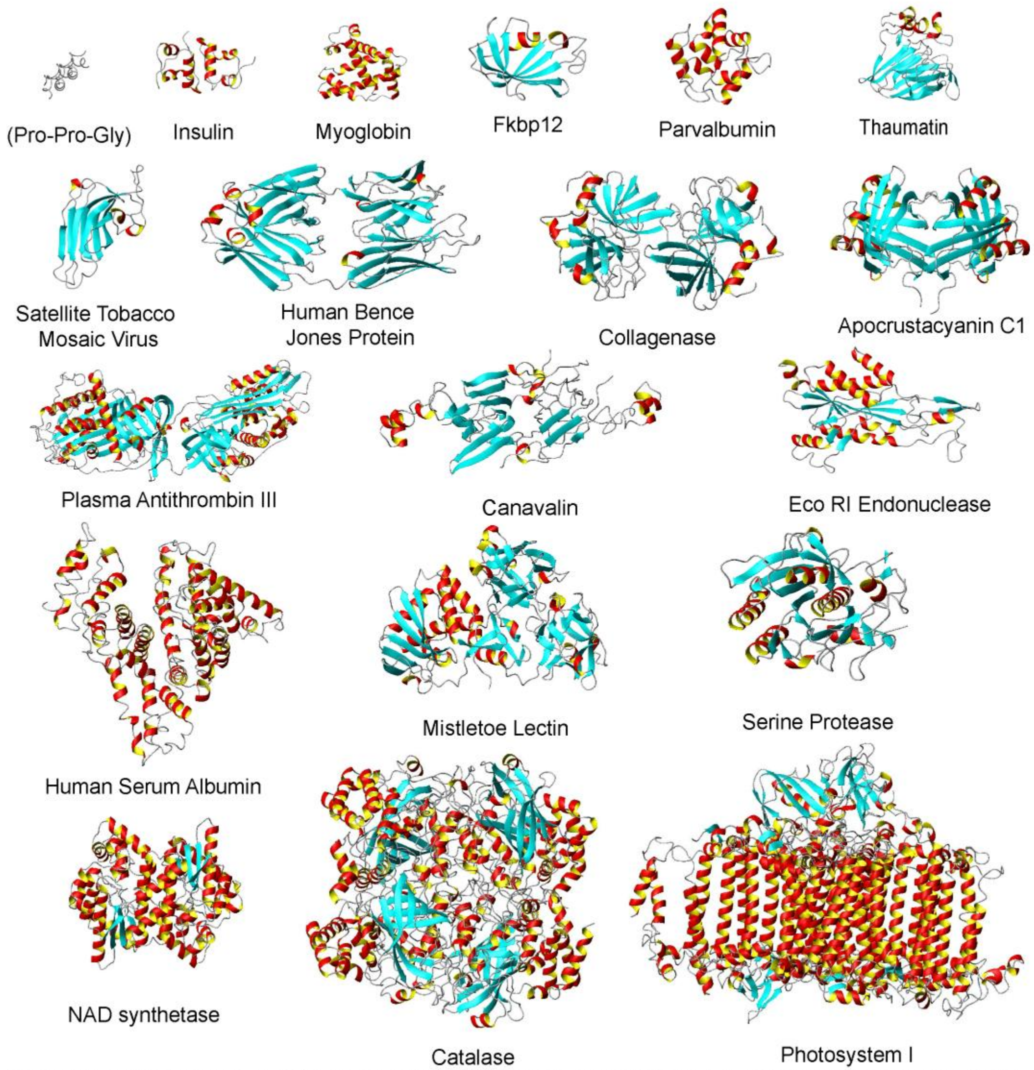


Improvements seen from microgravity samples (same reference as previous figure)



New and Improved Published Macromolecular Structures Resulting from Microgravity Research



References. (Pro-Pro-Gly) - Berisio 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, 982- 988; Fkbp12 - Wilson et al., Acta Cryst D. (1995) 51, 511-521; Parvalbumin - Declercq et al., Protein Science (1999) 8, 2194- 2204; Thaumatin - Sauter et al. Proteins: Struct, Funct, Gener. (2002) 48, 146- 150; Satellite Tobacco Mosaic Virus - Larson et al., J. Molecular Biology (1998), 277, 37-59; Human Bence Jones Protein - Terzyan et al., J. Mol. Recog. (2003) 16, 83-90; ; Apocrustacyanin - Habash et al, Acta Cryst D. (2003), 7, 1117-1123; Plasma Antithrombin III, Skinner at al., J. Molecular Biology (1997), 266, 201- ; Canavalin - Ko et al., Acta Cryst D. (2000), 46, 411-420; Collagenase - Broutin-L'Hermite et al. Acta Cryst D. (2000) 56, 376-378; Eco R1 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, Symersky 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 lysozyme - 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.

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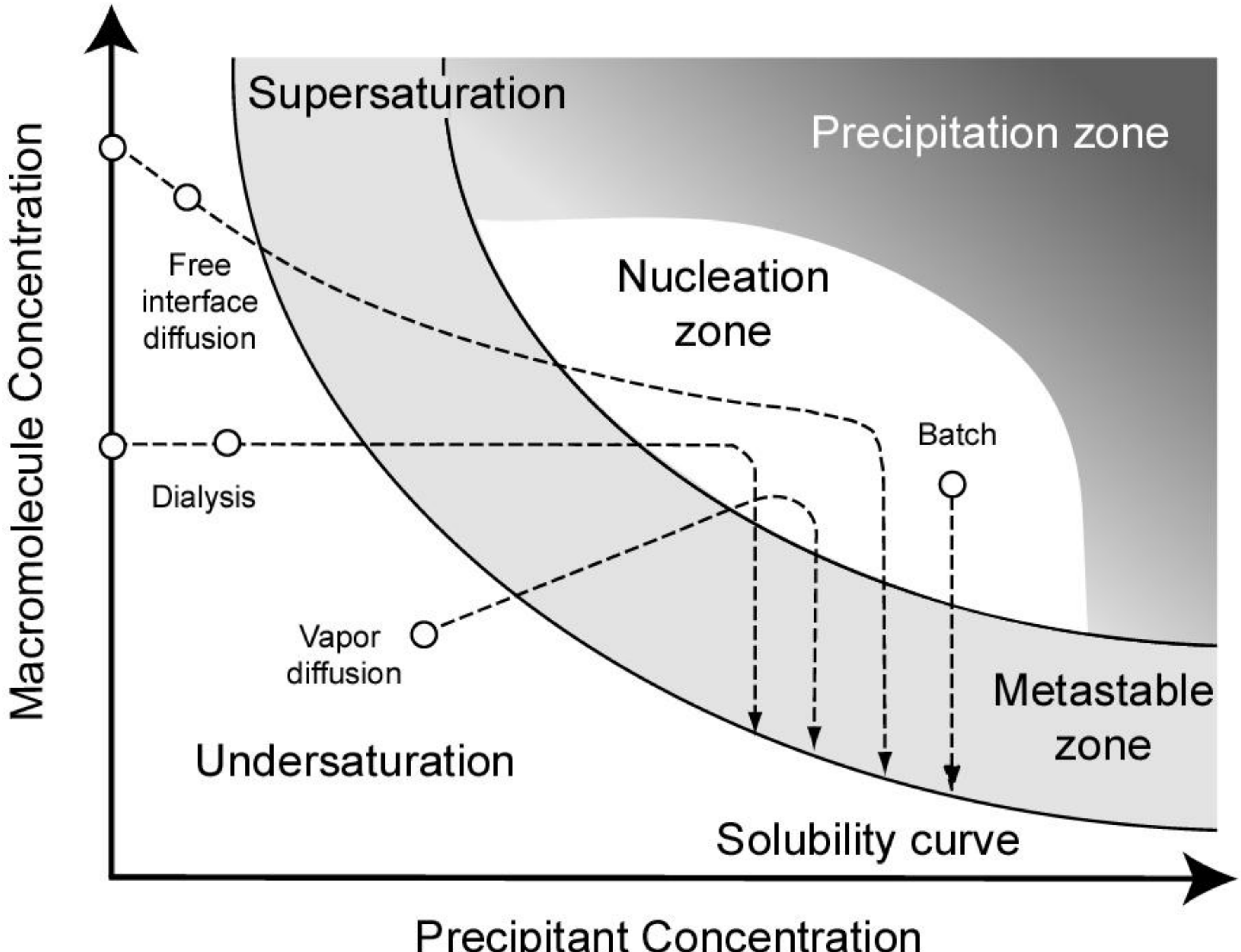
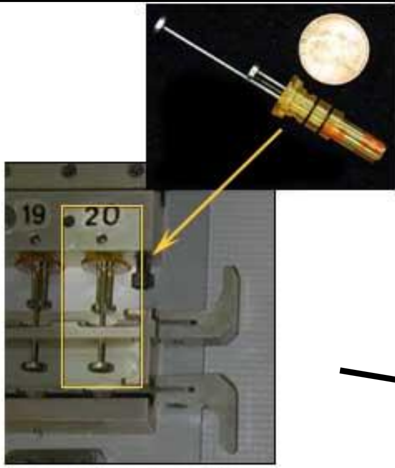
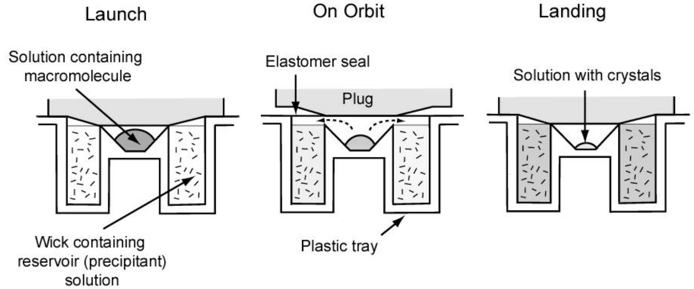

Macromolecule Concentration
Supersaturation
Precipitation zone
Free
interface
diffusion
Nucleation
zone
Batch
Dialysis
Vapor
diffusion
Metastable
zone
Undersaturation
Solubility curve
Precipitant Concentration

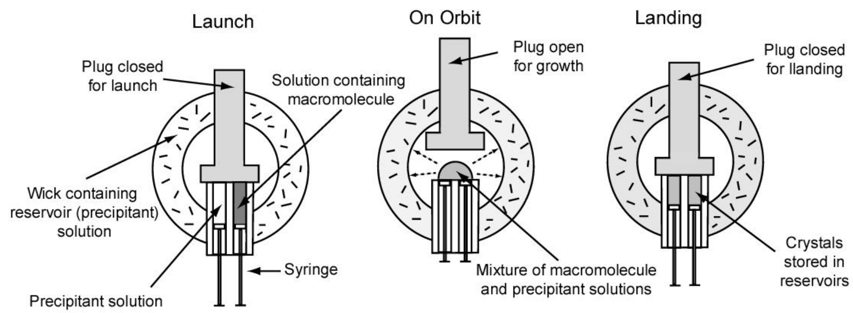


Top view of VDAs housed in a tray

(a) PCAM - Protein Crystallization Apparatus for Microgravity

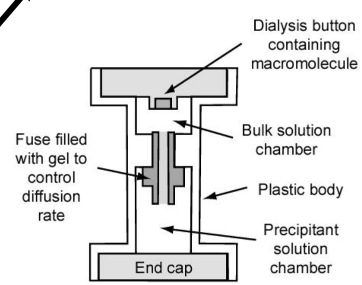


(b) VDA - Vapor Diffusion Apparatus

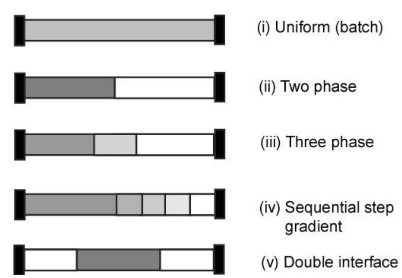


DCAM trays shown in Single-locker Thermal Enclosure System (STES)

(c) DCAM - Diffusion-controlled Crystallization Apparatus for Microgravity



(d) EGN - Enhanced Gaseous Nitrogen Dewar

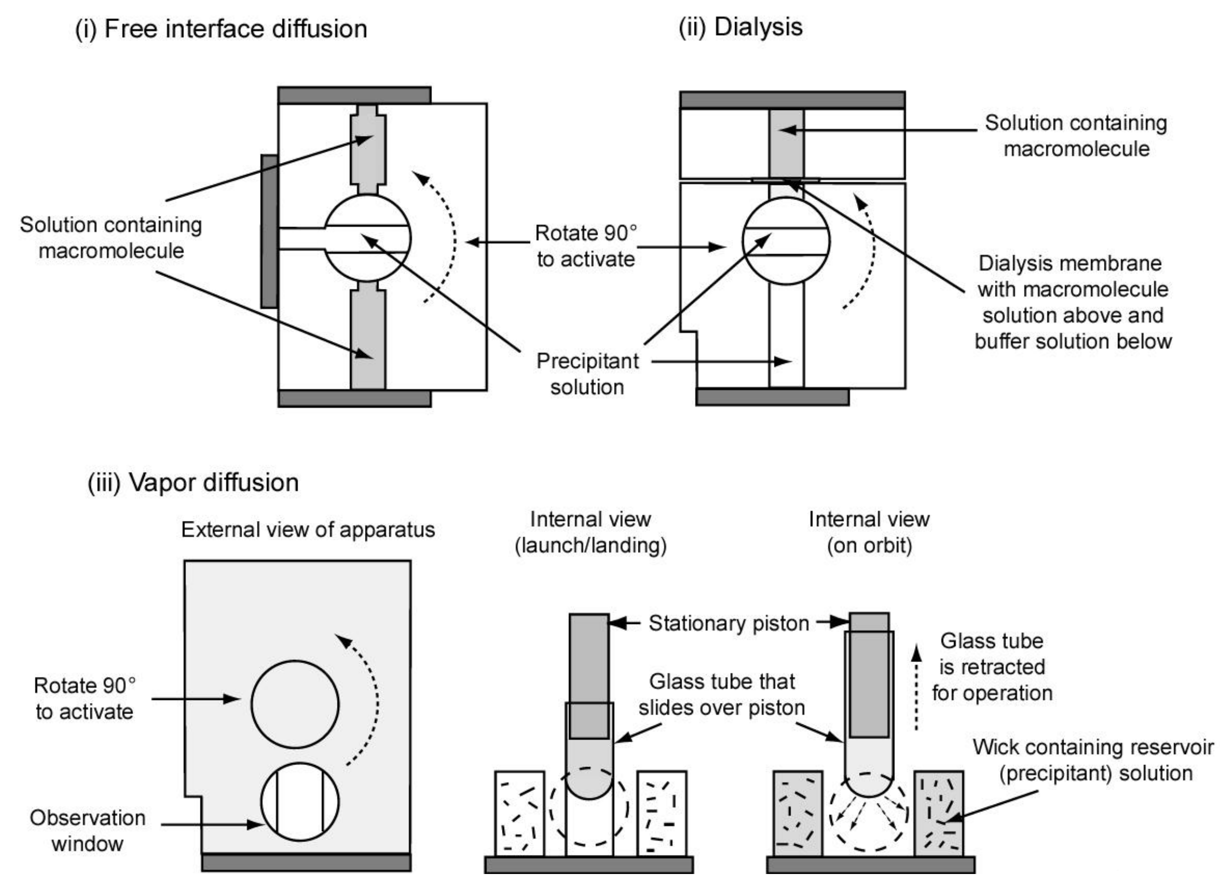


PCAM trays with 7 experimental cells (top).

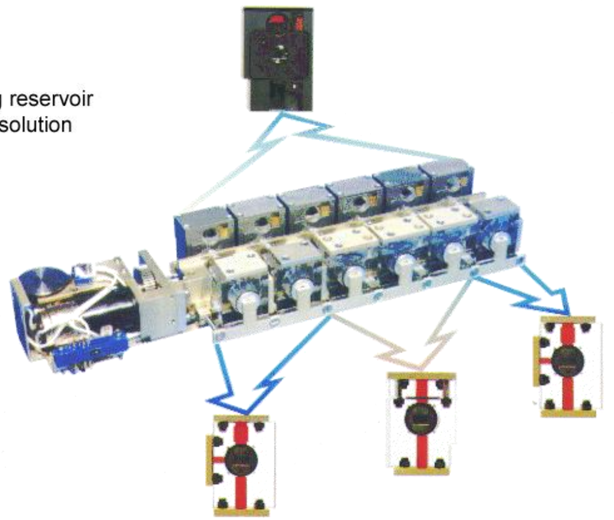
Nine trays are housed in one cylinder (center).

Six cylinders fit in a thermal carrier and are housed in an EXPRESS rack on ISS (bottom).

(e) APCF - Advanced Protein Crystallization Facility



Experiments can be imaged. A total of 48 experiments are accommodated in a temperature controlled incubator.



APCF is a single facility that supports several crystallization methods.

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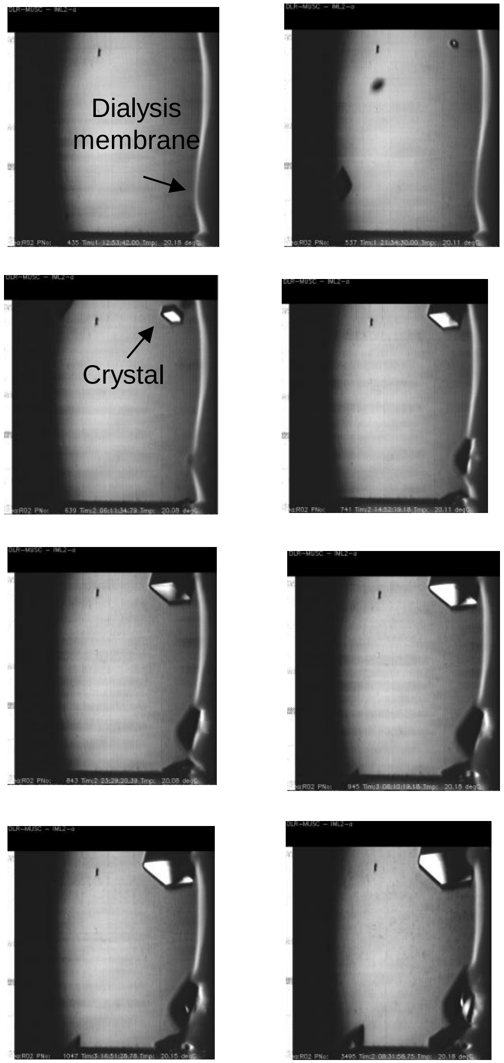
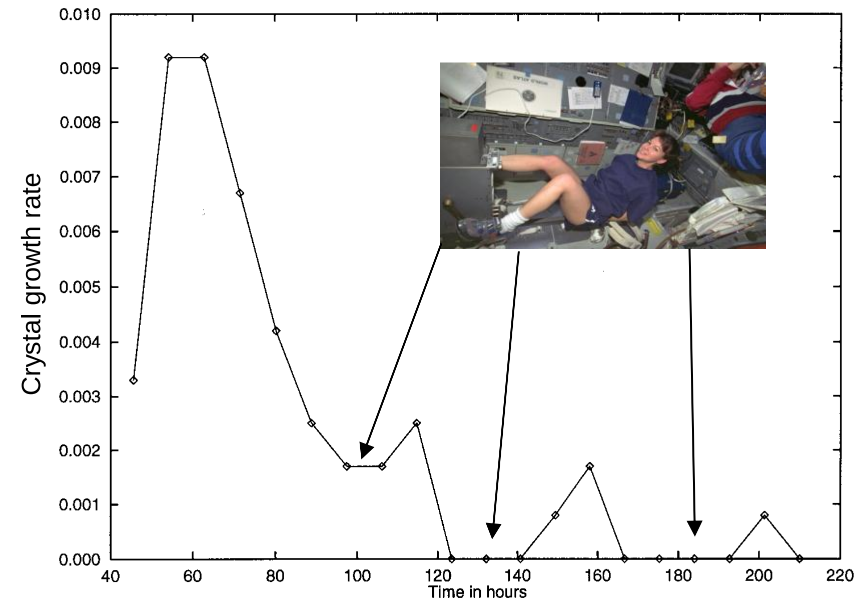
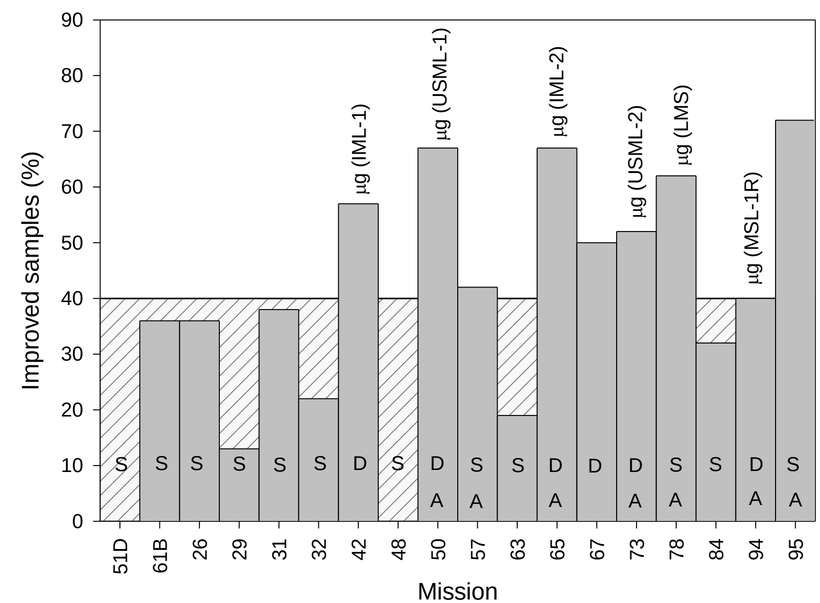


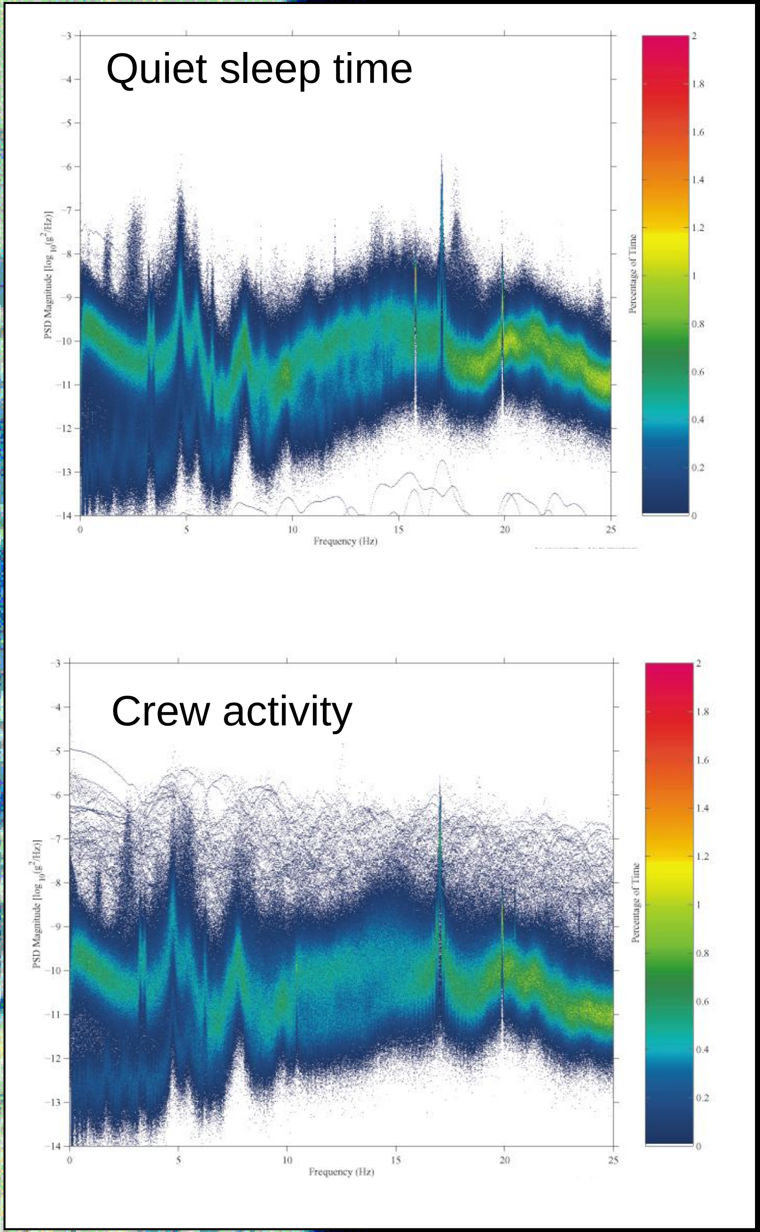
Single crystal imaged over time on STS 65. Snell at al. (1997)

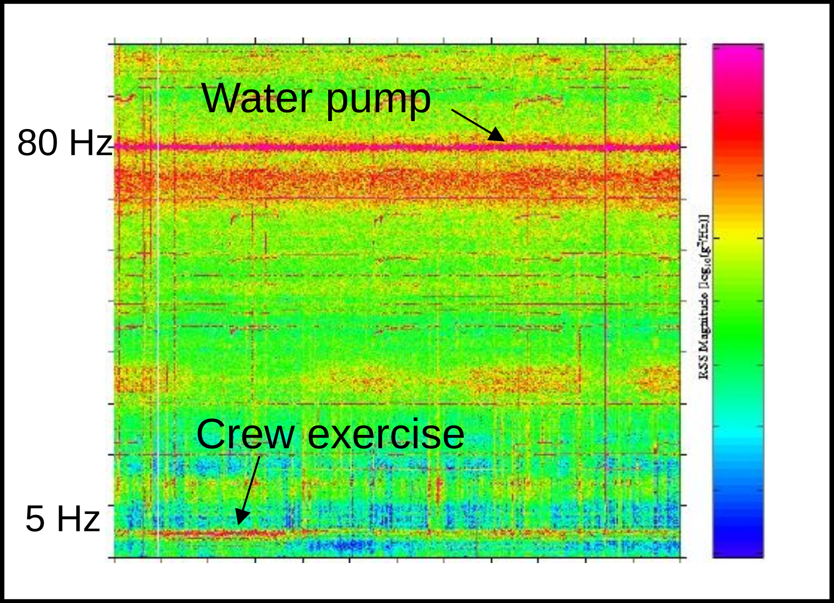




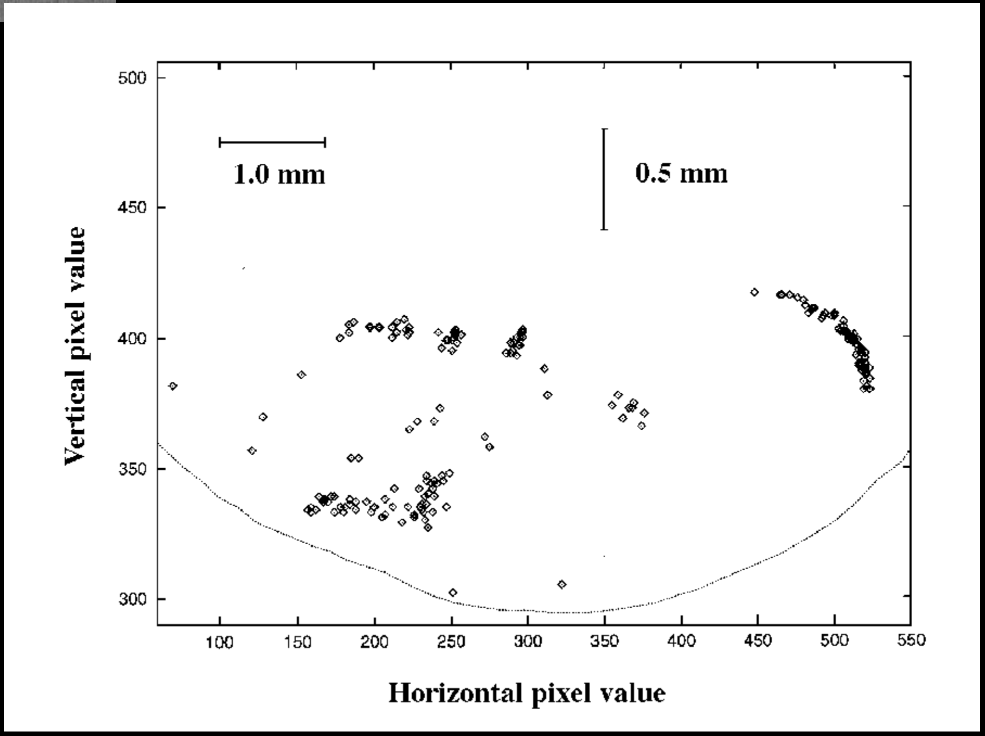
Noise

- The microgravity acceleration environment is noisy.
- This noise can be seen in the crystal growth experiments.
- We do not want this noise to mask our growth rate experiments.
- We need to measure the noise during the experiment to correlate it with any unexpected observations





Marangoni Convection



In the vapor diffusion case Marangoni convection occurs due to surface tension differences. This effect is masked on the ground.

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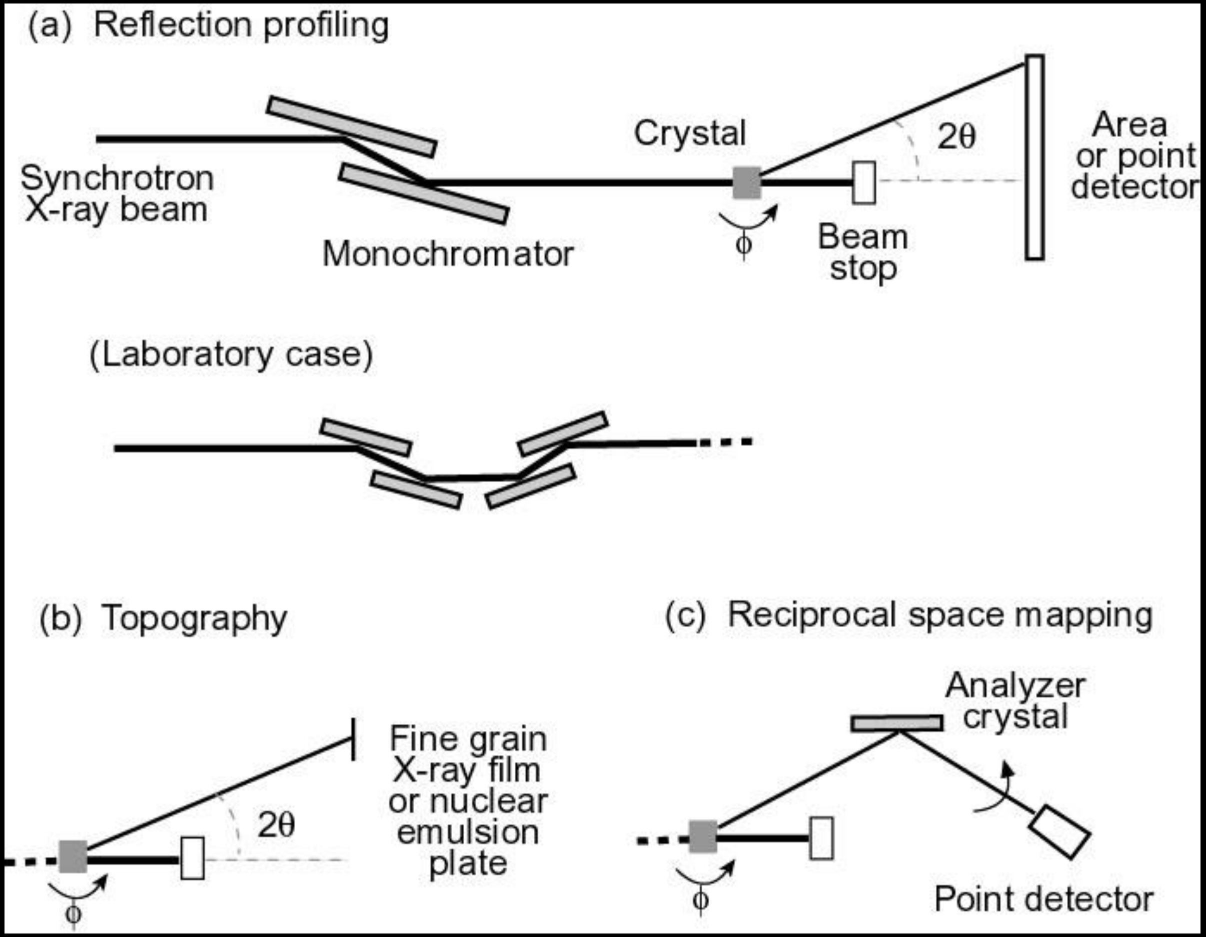
Techniques to study long range order

(a) Reflection profiling is very similar to structural data collection except that the beam has to be conditioned (more on that later).

(b) Topography images the reflection in a still.

(c) Reciprocal space mapping probes the interaction of the Ewald sphere with the reciprocal lattice point in two or three dimensions.



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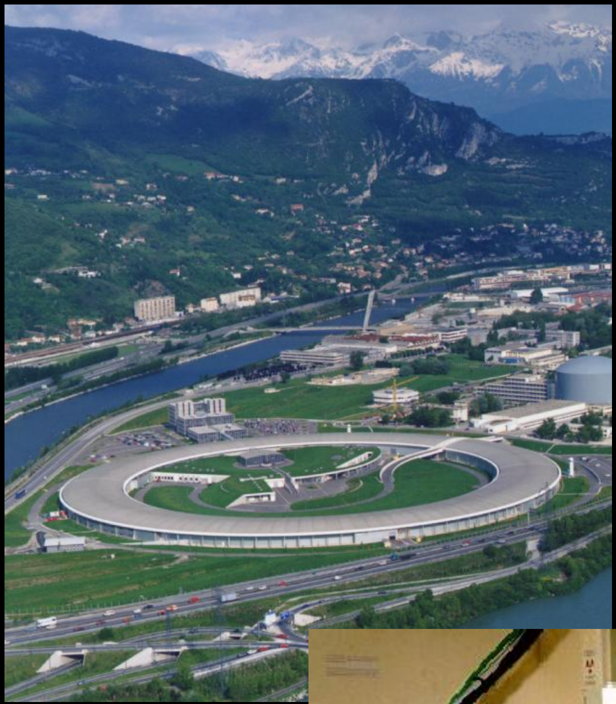
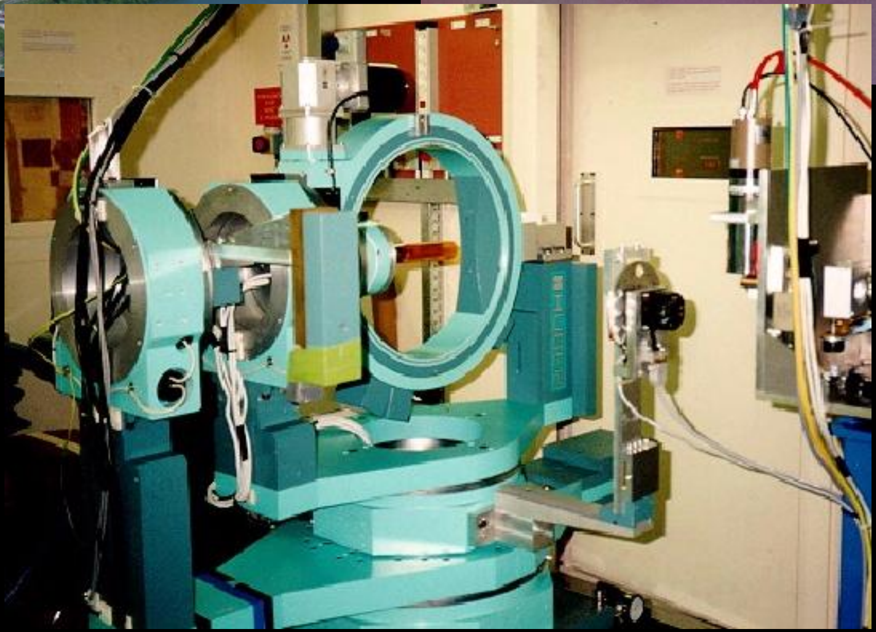


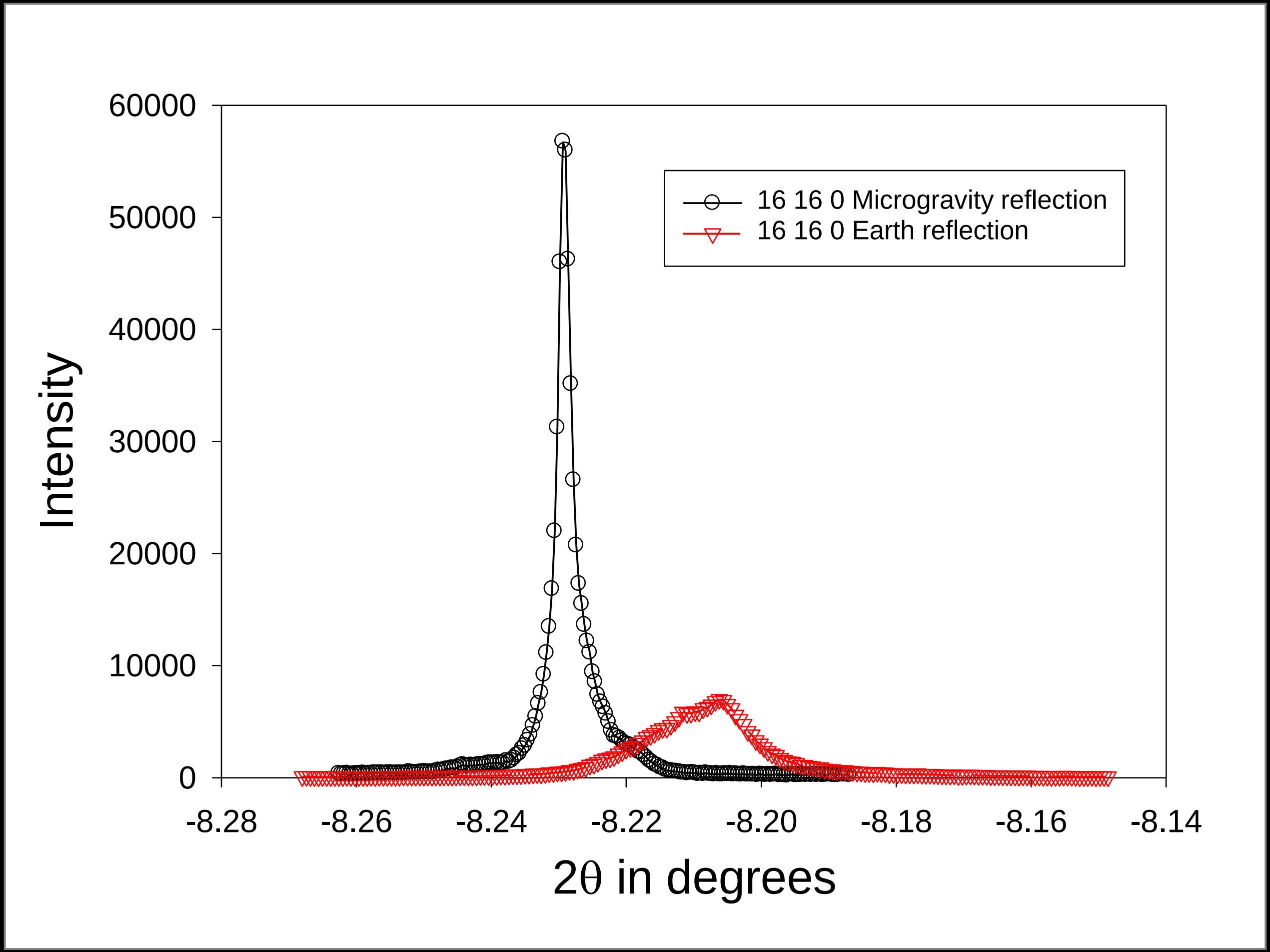


Lysozyme grown on STS-65.

Studied at the Swiss/Norwegian beamline of the European Synchrotron Radiation Facility in Grenoble France.

Highly parallel, monochromatic synchrotron radiation used,

Lysozyme rocking widths



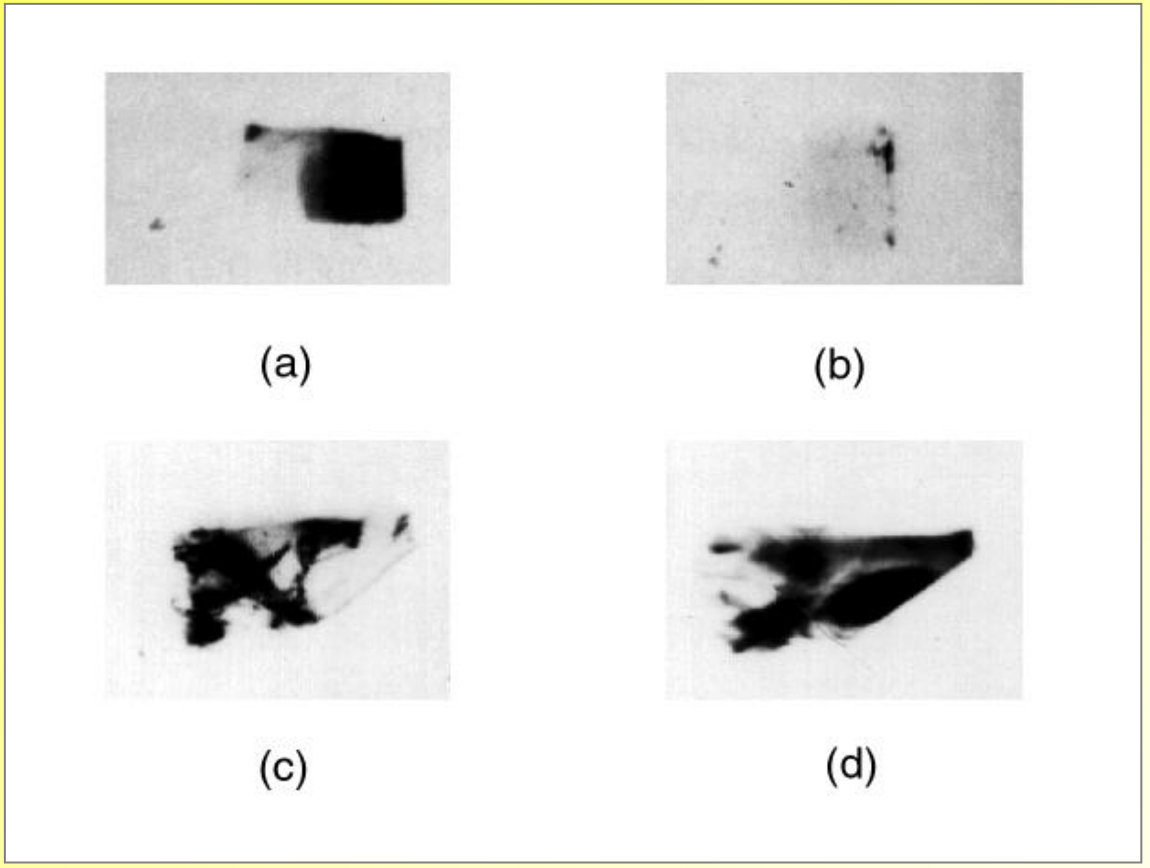
Identical reflections from microgravity and ground grown lysozyme.

Eight times increase in signal to noise.

The larger illuminated volume only accounted for a doubling.

Microgravity 0.0023 degrees, ground 0.0130 degrees.

)



X-ray topographs

Evidence for the domain makeup of crystals.

Each is a high resolution image of an individual reflection

From Snell, Borgstahl and Bellamy, *"Methods in Enzymology, Macromolecular Crystallography Part C".* Edited by Charlie Carter and Robert Sweet – to be published

Each topograph is a greatly magnified image of a reflection. In (a) and (b) the crystal is 1.1 mm by 0.9 mm in projection and defined regions are seen at the different reflections of (a) and (b). Some scattering is also seen on the crystal edges, probably due to mounting. In (c) and (d) the crystal is 1.5 mm by 1.1 mm in projection. In this case an array of domains is seen separated by a boundary layer. The different reflections (c) and (d) illustrate a region in the lower right of the crystal coming into the Bragg diffracting condition at the current orientation.

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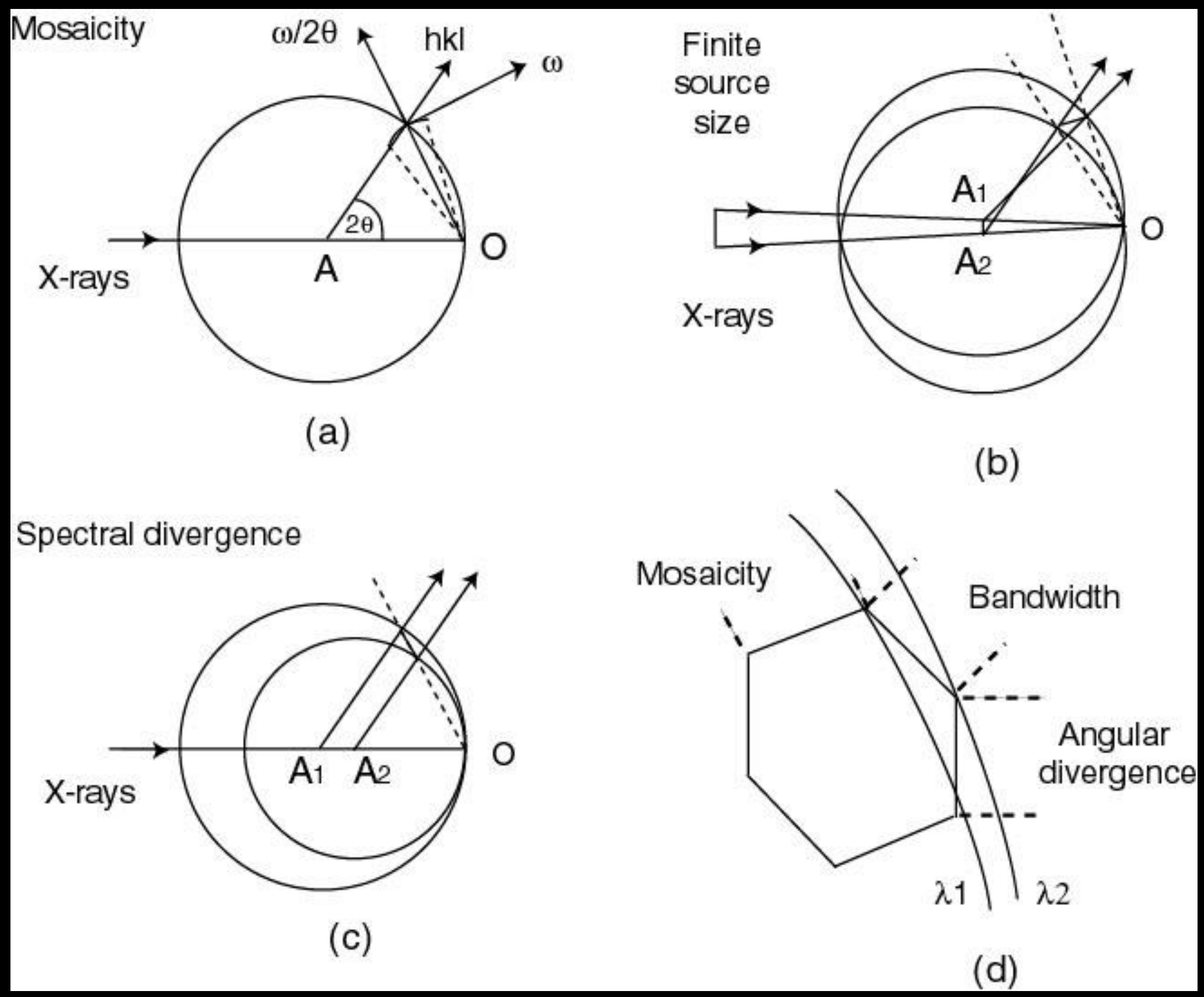
Factors that influence the reflection profile

The reflection profile is a convolution of a number of factors in the experiment and their interaction with the reflection through the Ewald sphere.

The mosaicity is just one of these factors and the remaining factors have to be deconvoluted out.



Deconvolution is the key to the technique:

Deconvoluting the measured data

Spectral

$$\left|\phi_R\right| = \sqrt{L^2 \varsigma^2 {\gamma_h}^2 + {\gamma_v}^2} + d^* L \cos\theta_{hkl} \left[\eta + \left(\frac{\delta\lambda}{\lambda} \right) \tan\theta_{hkl} \right]$$

Measured rocking width

Horizontal dispersion

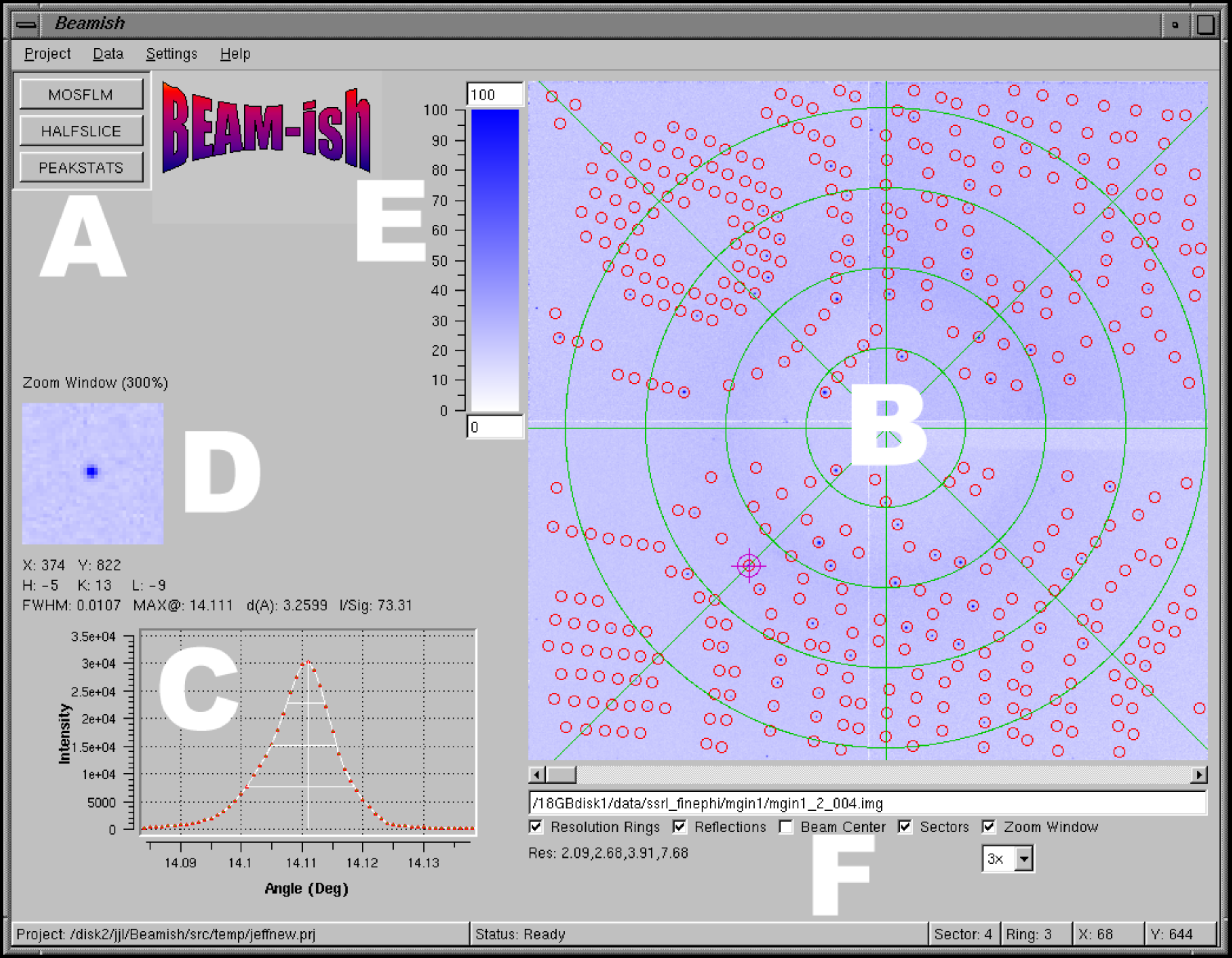
Vertical dispersion

True mosaicity

To measure the mosaicity, η, record data in fine slices, 0.001 degree, minimize vertical and horizontal divergence (synchrotron radiation) and monochromate the beam.

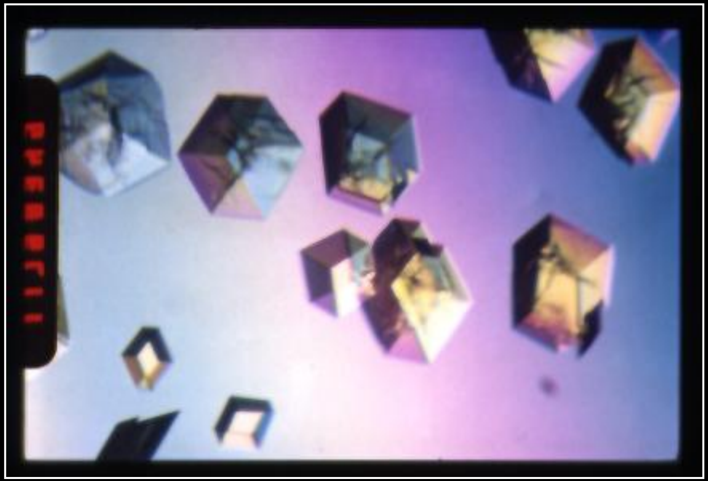
See Bellamy, H. D., Snell, E. H., Lovelace, J., Pokross, M. and Borgstahl, G. E. O. "The High Mosaicity Illusion: Revealing the True Physical Characteristics of Macromolecular Crystals" Acta Cryst. D56, 986-995 (2000).

Data Processing with BEAM-ish



Lovelace et al., J. Appl. Cryst. 33, 1187-1188, 2000

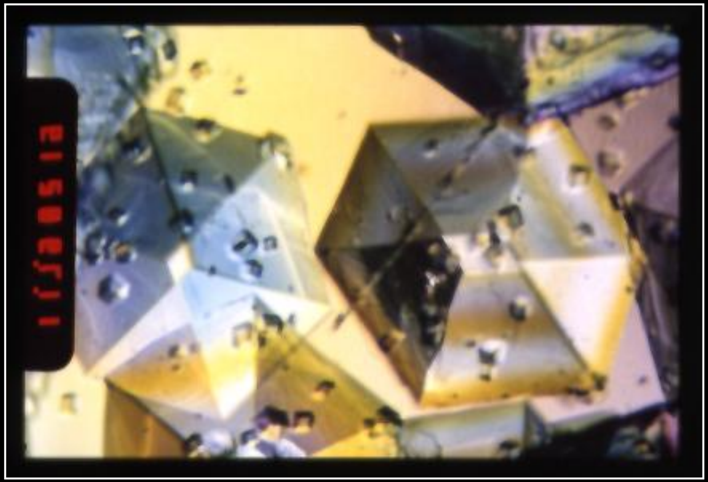
Studies on insulin



Images to same scale.

Ground:

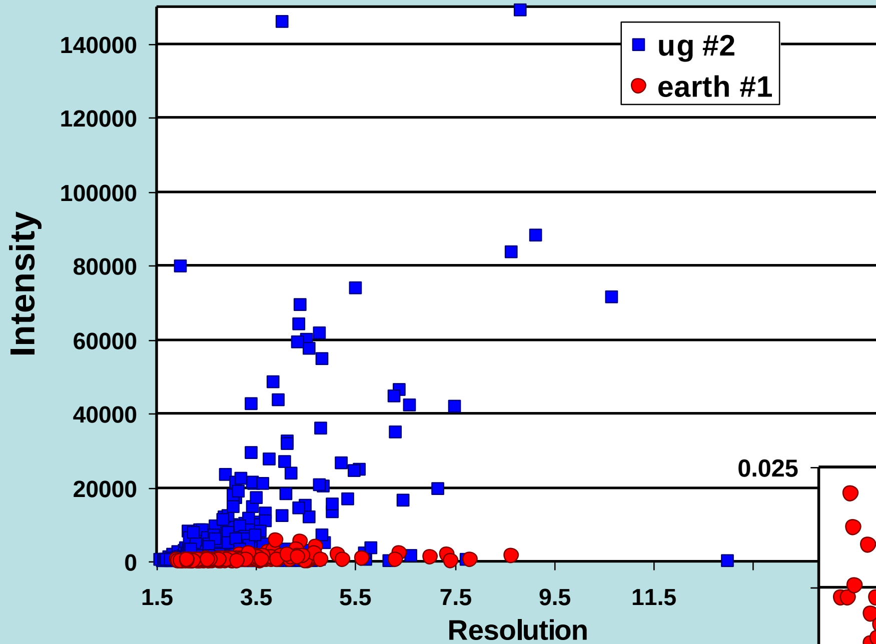
Sedimentation onto the bottom. Clumping of crystals.



Microgravity:

Free floating, unsedimented. had consistently larger diffracting volume > 2 mm in each dimension (34 times larger on average)

From STS-95. Borgstahl, G.E.O., Vahedi-Fardi, A., Lovelace, J., Bellamy, H. & Snell, E.H. Acta Cryst, D57, 1204-1207 (2001).



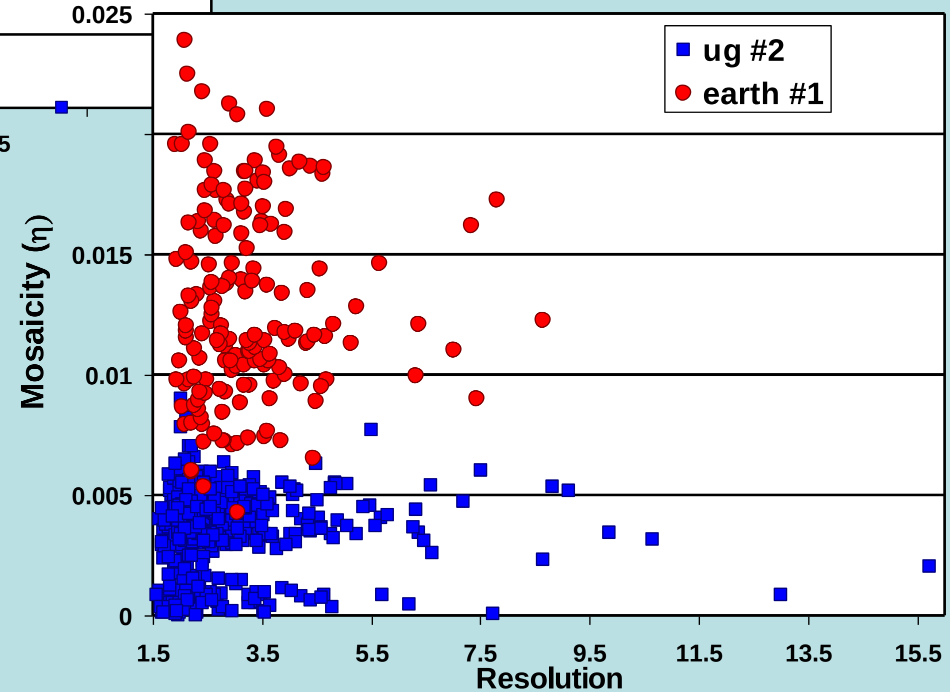
Microgravity Blue

Ground Red

Structural, short range improvement

Physical, long range improvement.

The worst microgravity and best ground crystal data are shown.



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- Crystallization experiments are small volume, have low mass, are simply operated and can have high scientific and commercial return.
- There have been a number of demonstrated improvements in microgravity.
- The quality of the microgravity environment is critical.
- Large domains result in the crystal.
- The mosaicity is reduced.
- Consequently resolution is increased if the reduced mosaity is exploited.
- Microgravity can have an impact for structural crystallography.

Acknowledgements

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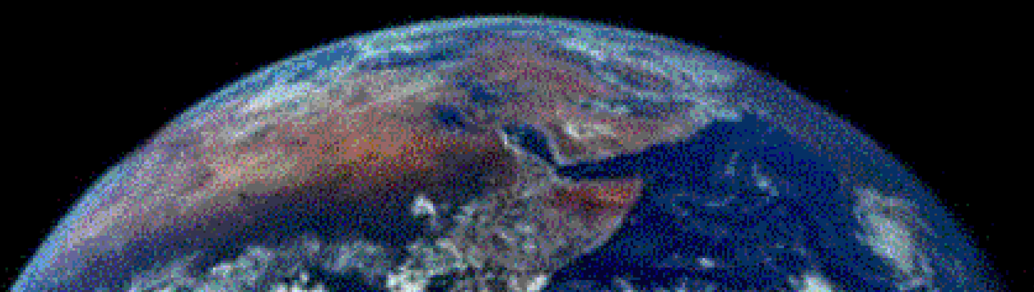

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