



SCIENCE DIRECTORATE Biotechnology Science Laboratory **Dr. Edward Snell**

NASA Laboratory for Structural Biology Marshall Space Flight Center Huntsville AL. Eddie.snell@msfc.nasa.gov (256) 544 5570

A visit to the Structural Biology Laboratory at Marshall Space Flight Center

Microgravity Crystallization

Edward Snell

NASA Laboratory for Structural Biology

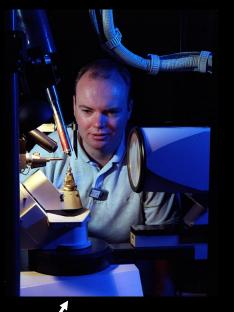


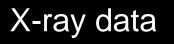
Introduction

Order in the crystal Why microgravity History of Results Methods of growth Apparatus During mission X-ray analysis Case study: Lysozyme Insulin Summary



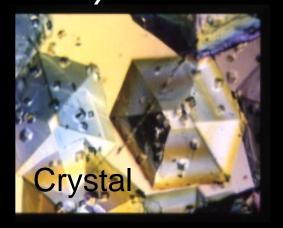
Crystallography process

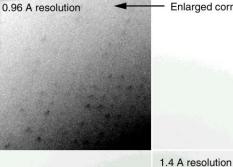




Enlarged corner of detector

2.4 A resolution



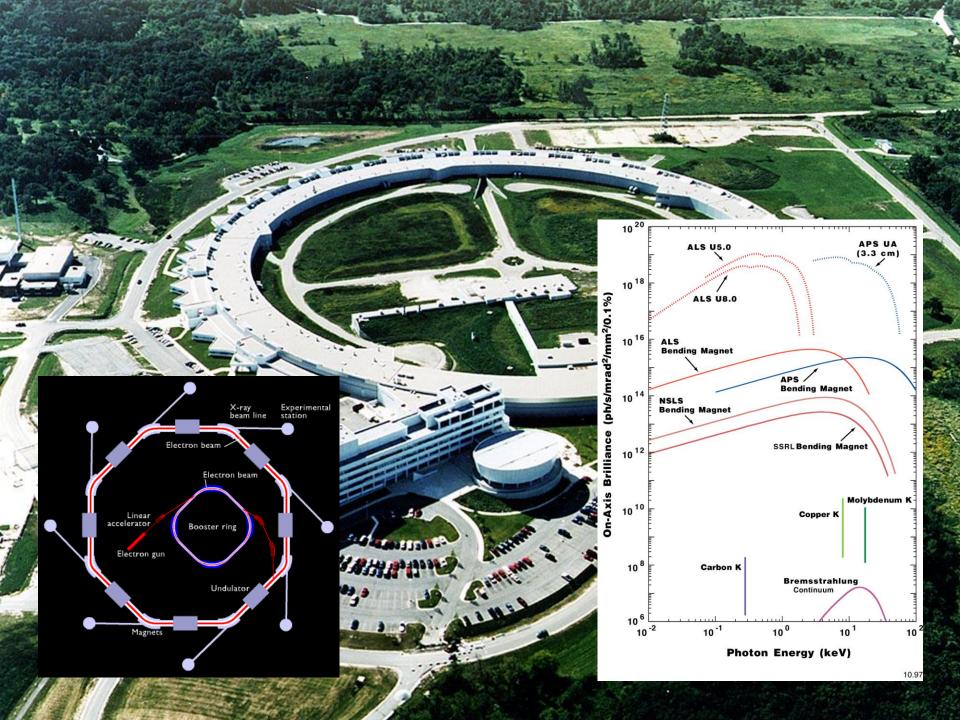


Overloaded data collected in later low resolution fast pass



Target

for drug



Introduction

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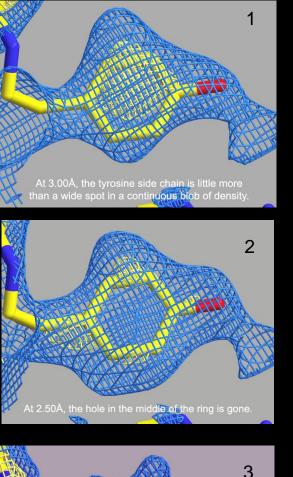
Order in a crystal

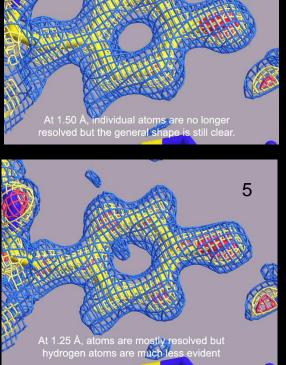
- An atom only contributes to the intensity of the diffraction spot if its disorder relative to symmetryrelated atoms is small compared to the resolution of the spot.
- The internal order in a crystal can be characterized by a correlation length, *i.e.* the distance over which the two atoms in a unit 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).

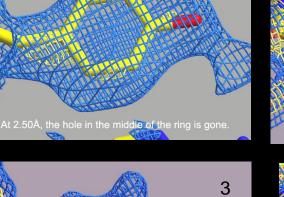


Short and long-range order

- 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.

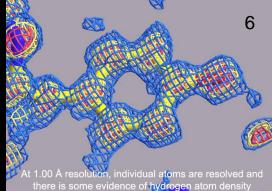






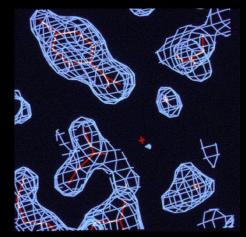
At 2.00Å, the shape is still there but the hole

in the middle of the ring is much smaller

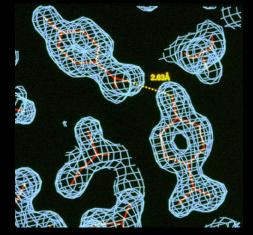


Quality (Resolution)

Low resolution ground



High resolution microgravity



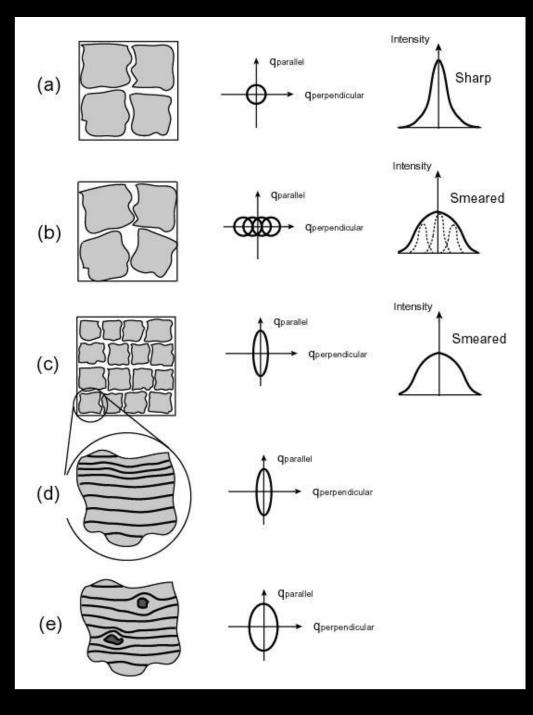
Sean Parkin. UK

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.



Introduction Order in the crystal

Why microgravity?

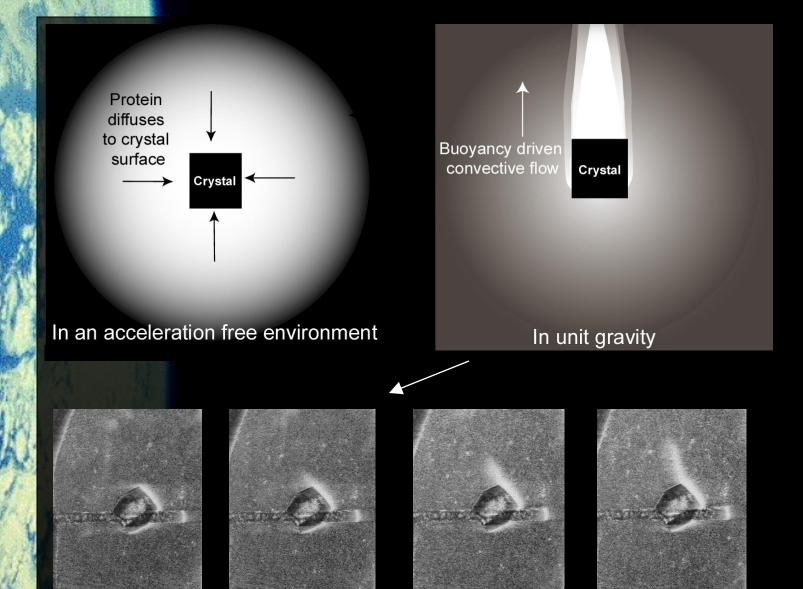
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So why is NASA interested?

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



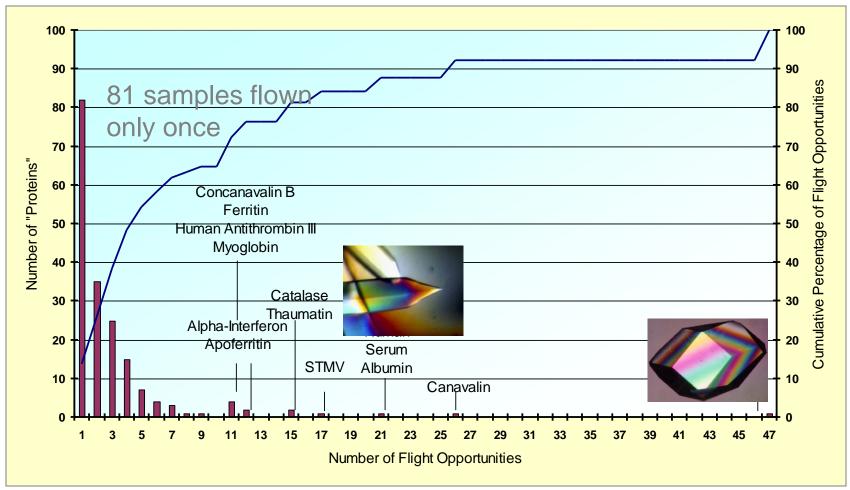
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History of Results

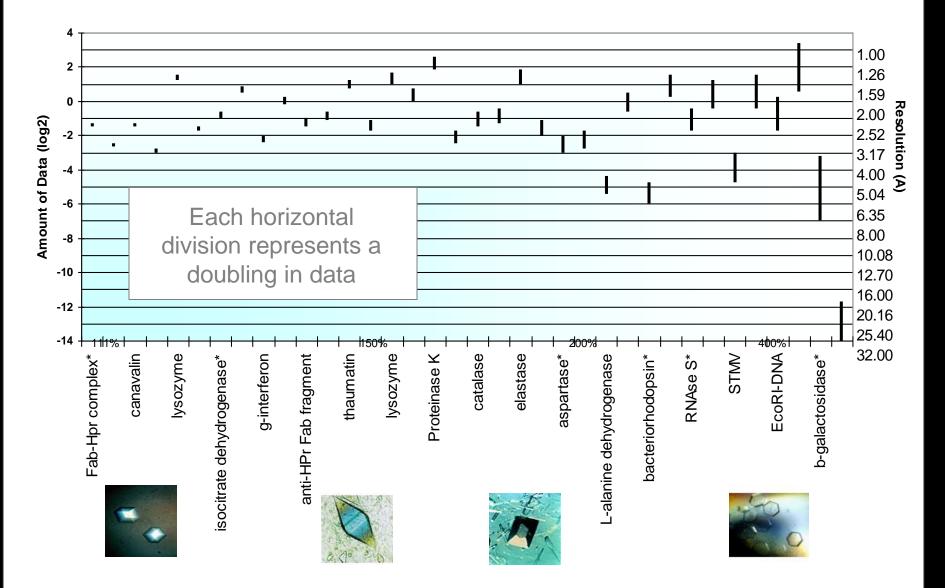
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Over 185 different macromolecular samples have flown. Some are frequent flyers, others have flown only a limited number of times



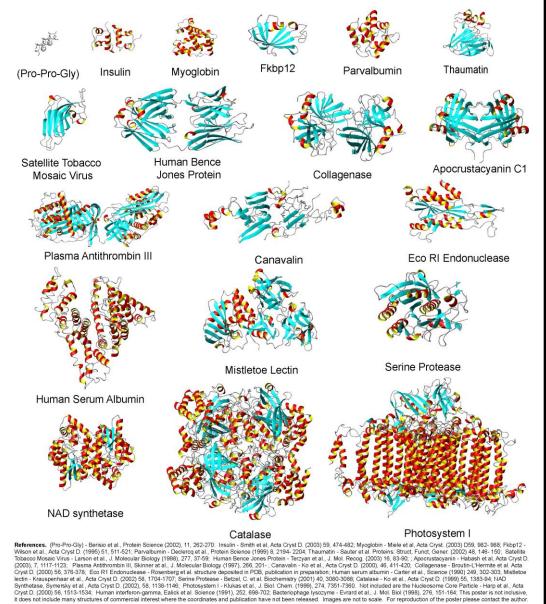
Microgravity and Macromolecualr Crystallography: Kundrot, C.E., Judge, R.A., Pusey, M.L., & Snell, E.H. Crystal Growth and Design. Crystal Growth and Design, 1, 87-99 (2001).



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



New and Improved Published Macromolecular Structures Resulting from Microgravity Research



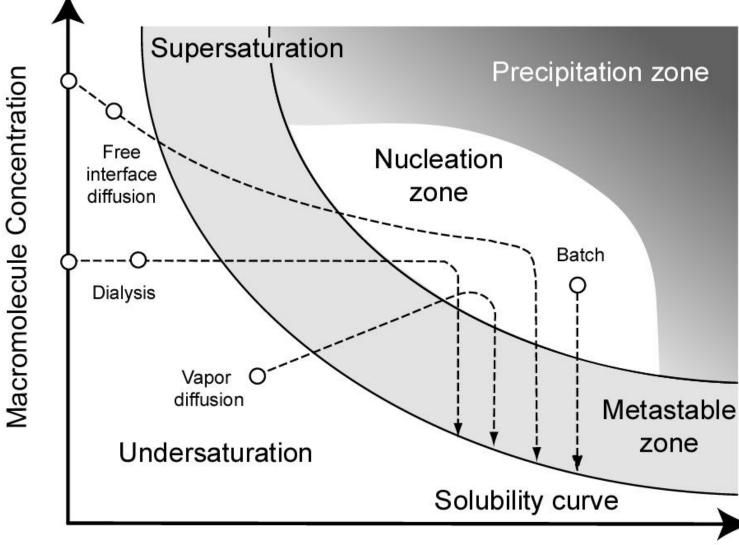
Edward H. Snell, 2003.

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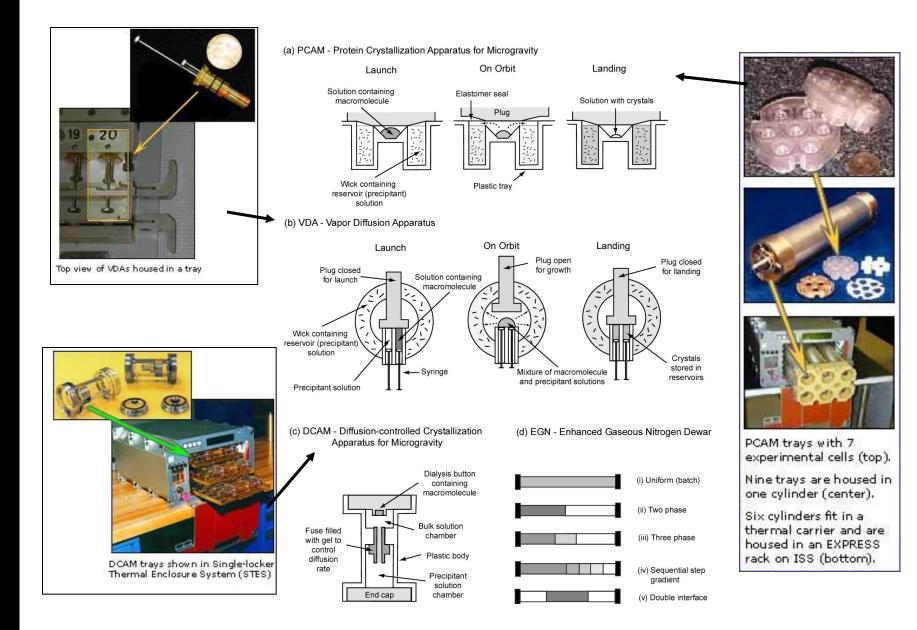
Methods of growth

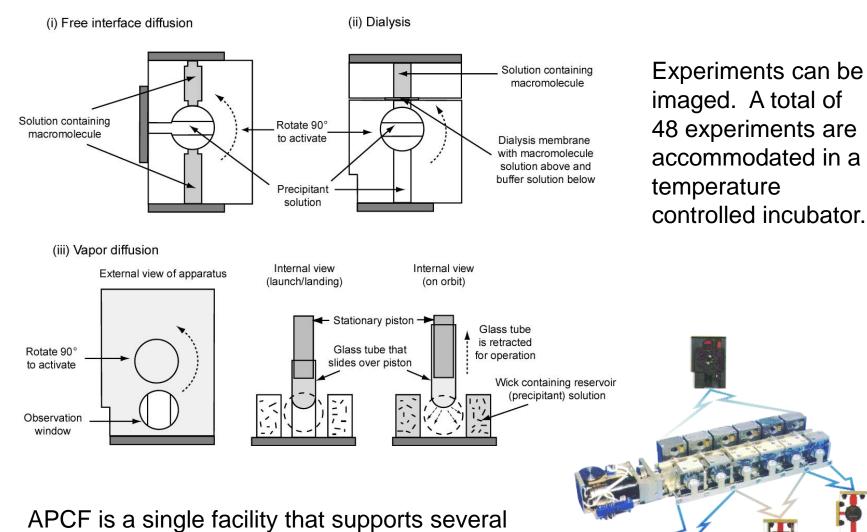
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Precipitant Concentration





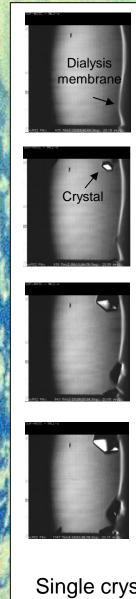
crystallization methods.

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During the mission

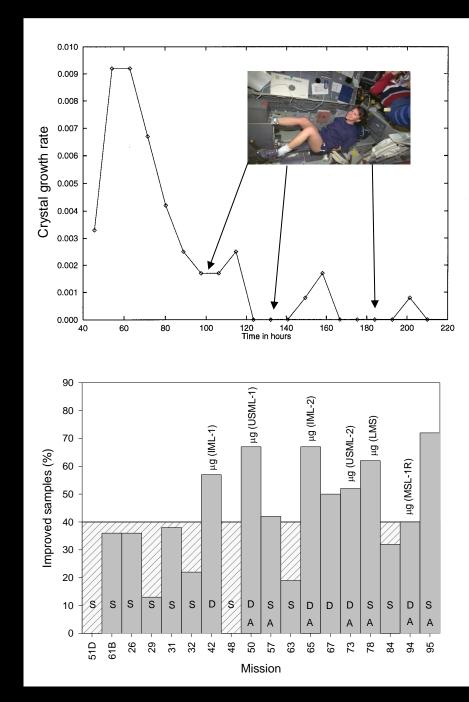
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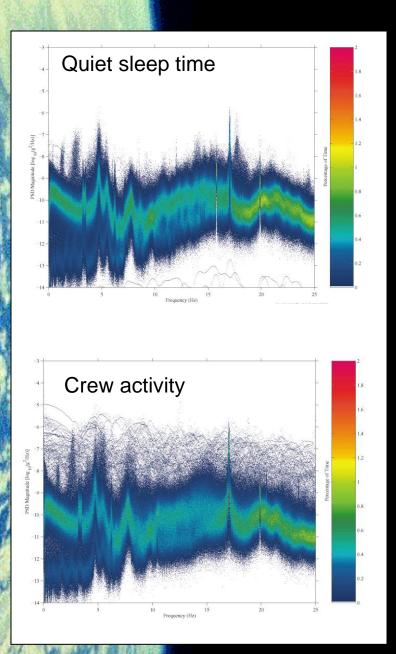






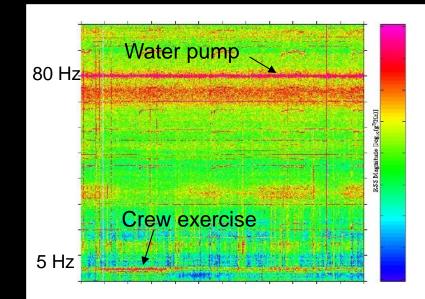
Single crystal imaged over time on STS 65. Snell at al. (1997)





Noise

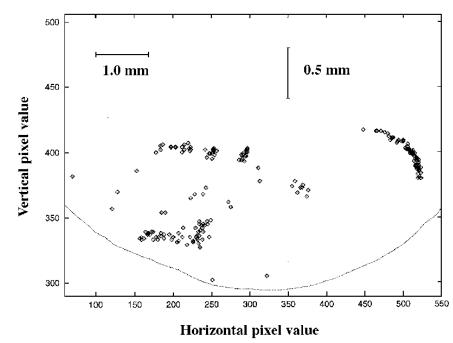
- The microgravity acceleration environment is noisy.
- This noise can be seen in the crystal growth experiments.
- We do not want this noise to mask our growth rate experiments.
- We need to measure the noise during the experiment to correlate it with any unexpected observations



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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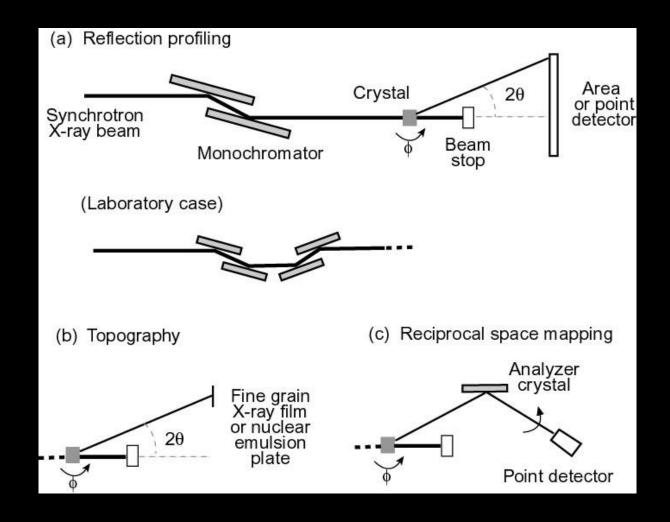
X-ray analysis

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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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Case study: Lysozyme

Insulin Summary



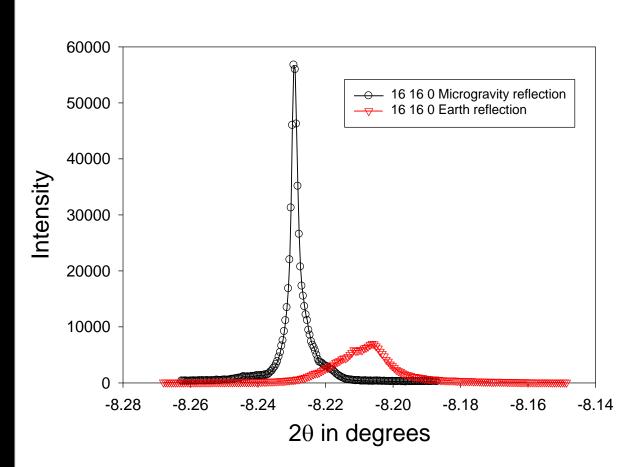


Lysozyme grown on STS-65.

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

Highly parallel, monochromatic synchrotron radiation used,

Lysozyme rocking widths

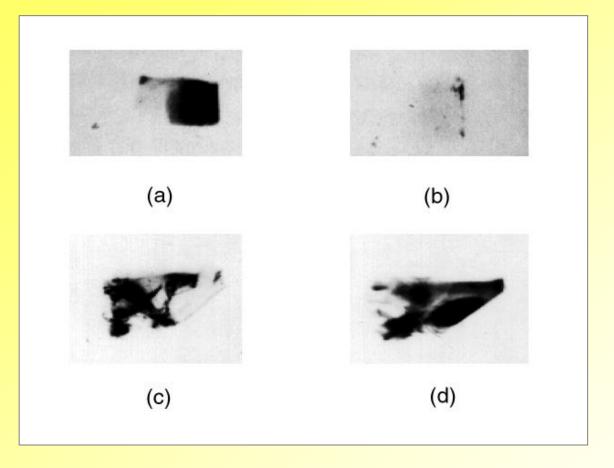


Identical reflections from microgravity and ground grown lysozyme.

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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Insulin

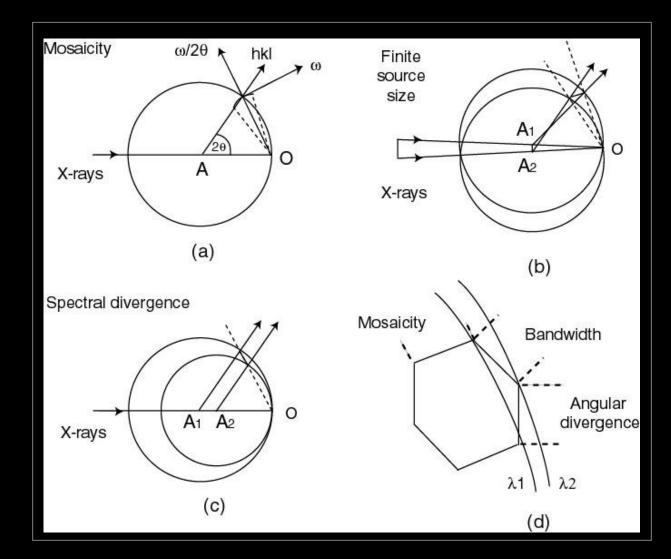
Summary



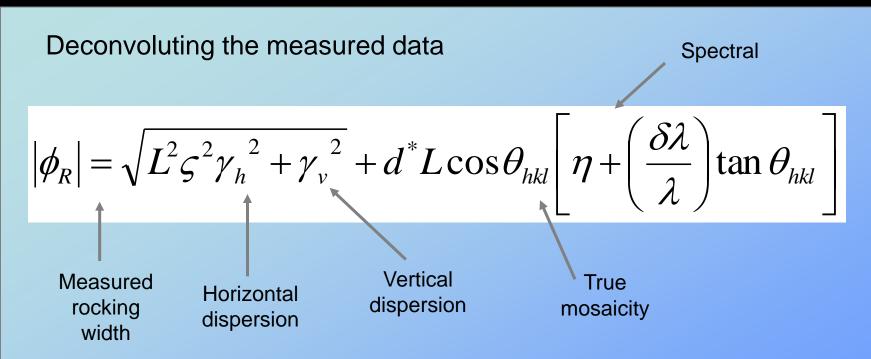
Factors that influence the reflection profile

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.



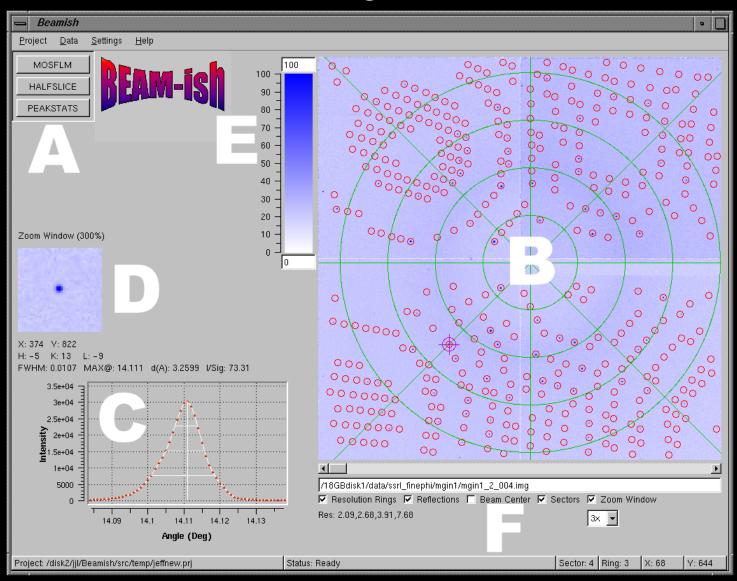
Deconvolution is the key to the technique:



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).

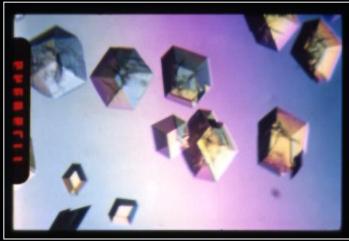
Data Processing with BEAM-ish



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



Studies on insulin





Images to same scale.

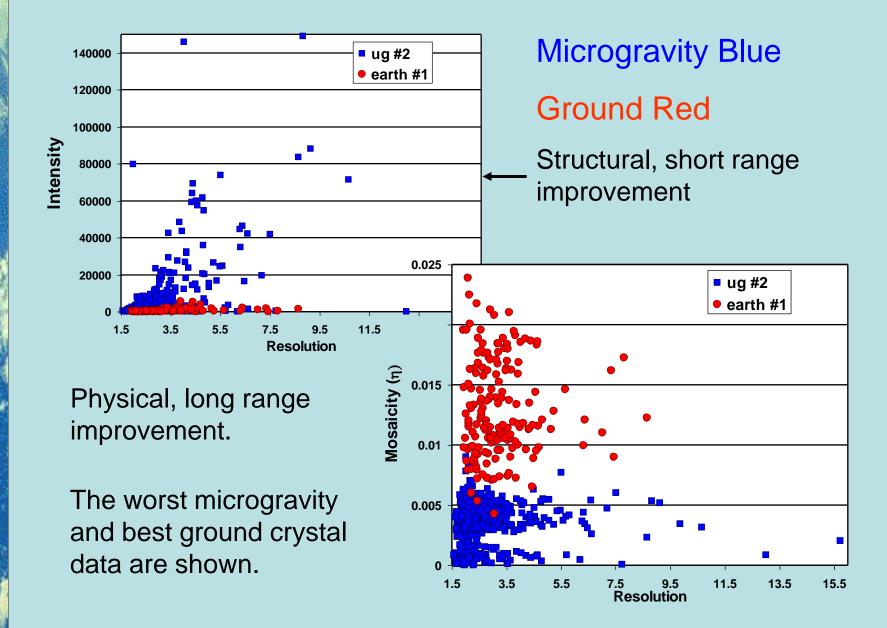
Ground:

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).



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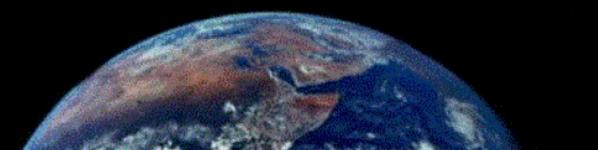


Summary

- 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

- Matt Pokross and Jeff Lovelace
- Walt Pangborn, Bob Blessing and Dave Smith
- Chris Nielsen and Andy Arvai
- Marc Pusey, Russell Judge and Craig Kundrot
- Marianna Long and Karen Moore
- John Helliwell, Titus Boggon and Susan Weisgerber
- NASA for funding support



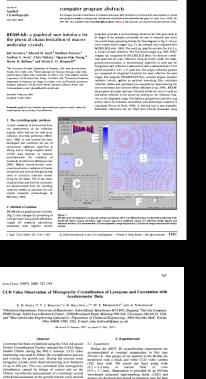
Electronic (pdf) reprints available

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computer program abstracts



*To shost correspondence should be addressed. Phone: (250)544. 2533. Face (25) 544-0352. E-mail: or algorator sectors for the colloquial and scientific senses. In the colloquial sense it means an 10.1021/g005511b CCC: 120.00 0 2001 American Chemical Society Published on Web 11/15/2000



	GROWTH		Improvements in lysozyme pr S. WERGERER and J. R. HELLT England, and E. WECKERT, K. (770) Keinertenen 12 Braded	WILL,* Chemility Dept	priment, University of	Manchester, Manchester M13 9P
ELSEVIER Journal of Crystal Growth 17	1 (1997) 219-225		(71f), Kaiserstrasse 12, Postfach	(Revived 28 Jab 1995)	new, Germany	
			Abstract			
CCD video observation of microgravity crystallization: apocrustacyanin C ₁ N.E. Chayen **, E.H. Snell ³³ , J.R. Helliwell *, P.F. Zagałsky * ***********************************			Abstract Microgramy offen as moviousness for them from is as abstrace of correct of the second second second second in the perfection of proteins crystals. For X-big JBPacther indices in these interpreting and the second second second with crystal gravels in the second second proves provide a systal as in the second proves provide a systal as an inter- ested second second second second second proves provide any second second second proves provide second second second proves provide second second second proves provide second second second fractional second	r protein crystallization tion and sedimentation microgravity conditions. The quality of crystals testized by a matuber of the resolution limit. By a a test case we show, Space Shattle missions, facure of three to four	Crystall	and a tightly 'silted-down' X-ray bea o allows a batch of crystals to be surray and class collections with a diffuscore et of the diffuscion patterns to be prod- with such fire socking widths requires small arguine step with combined so investite synchrotron beam, also of ve- litention and X-ray analysis
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1. Introduction	fusion has been monitored by CCD observation. The X-ray diffraction analysis on the resulting crystals is		& Stapelmann, 1992) as a standard crystallization experiments aboard the We base atilized dialysis liquid diffs within the APCF. The dialysis reacto	tool for microgravity NA3A Space Shuttle, nion for crystalization on each consist of two		(a)
Microgravity has been used as a growth mediam to operent sedimentation and neduce buoyancy forces within crystallization media. Crystals grown in mi- rogravity have been silenon to produce enhanced ignal to noise X-ray diffraction over their corres- ponding earth counterparts [1]. This is advantageous n collection of weak high resolution data. Several methods of microgravity crystallization are available a peesent; vageour diffraien, liquid effision.	described elsewhere [2]. The vagoes efflution apparatus consists of a drop- containing the protein solution which is separated by an air space from a suscrivit containing a concen- tualed precipitate solution. Ware its smallered from the protein solution to the precipitate solution via vopen-diffusion thereby increasing the superstant- tion in the protein solution and initiating ergualita- tion. The transfer of protein from solution to be		Displayers of the second se	survers separated by a set contains the protein robotion. The salt and cylindicial quartz glass. To activate the reactor bit is reached, so that Likewise the reactor is to the precision with 10 ¹⁰ or so unit cells clion can be evaluated lion.	04.10 År	5.00 Colored and
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¹ Composing autor. Fac. +64 171 589 0191. E-mail: cdtpstr4Eac.uk. ¹ Present address: NASA, Laboratory for Stratasel Biology. Code 8570, Bilg 4664, MSFC, Hautoville, Alabura 35812, USA.	erss, due to the depleted region, result in buoyancy forces and initiate convection which can be detri- mental to the growing crystal [4]. Microgravity greatly reduces these buoyancy forces allowing, it is thought [5:6], a more stable depletion layer to form		indiation is ideal for probing the most theoretical limit (Heilzivell, 1983), rathe by the X-ray beam divergence and a methods of measurement have been a of polytheorentic Lasse data and collec data. To explore the fine mesaicity value	activ of crystals in the rr than having it moded dispersion effects. Two atilized here: collection ition of monochromatic es expected, <i>i.e.</i> 0.0005 ¹¹	Fig. 1. The sensepts of 1 crystal. For rase (so the larger overall crys- the messicity or non- case (b) a simplified.	(b) (b) which interfect crystal and (b) a parties as number of smaller perfect blocks make u tal, the overall minalgament of such blocks in an updat, the overall minalgament of such blocks in an update of coloridation of the recking with the recking with typical upsneal size of 50 Å and a crystal is
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Investigating the Effect of Impurities on Macromolecule Crystal Growth in Microgravity Edward H. Snell,^{*2} Rassell A. Judge,¹ Lisa Crawford,¹ Elizabeth L. Forsythe,¹ Marc L. Pusey,¹ Michael Sportielle,⁵ Paul Todd,¹ Henry Bellamy,³ Jeff Lovelace,¹ John M. Cassunta-¹ and Gozia E. O. Borantabl⁰

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