Growth Rate Dispersion, a Predictive Indicator for Biological Crystal Samples that Improve in Microgravity

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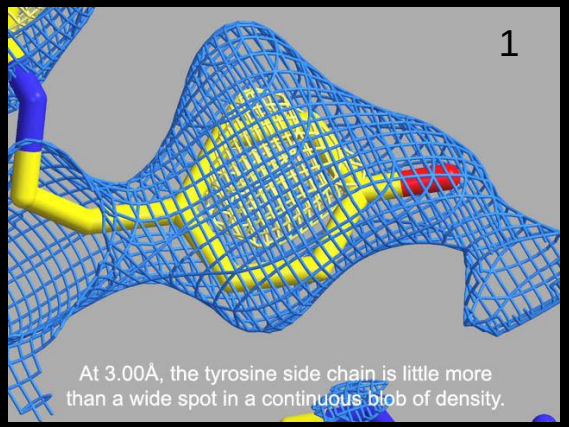
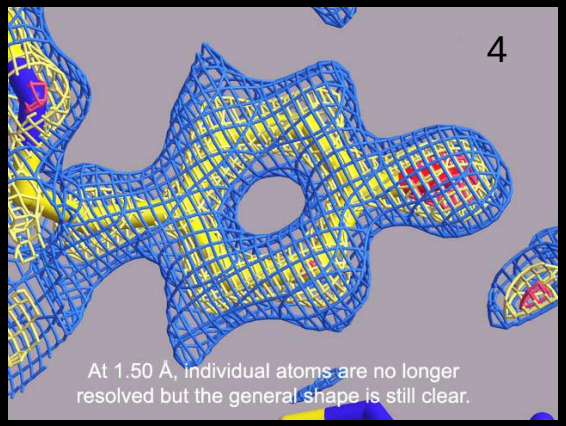
Why grow biological crystals in microgravity?

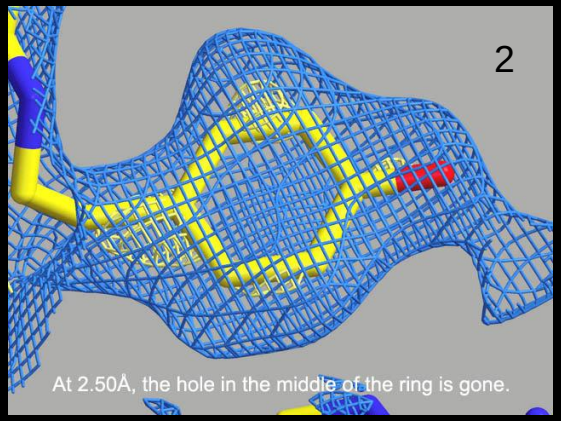
Most biological processes in particular those of health related interest (both on the ground and in space) occur at the molecular level.

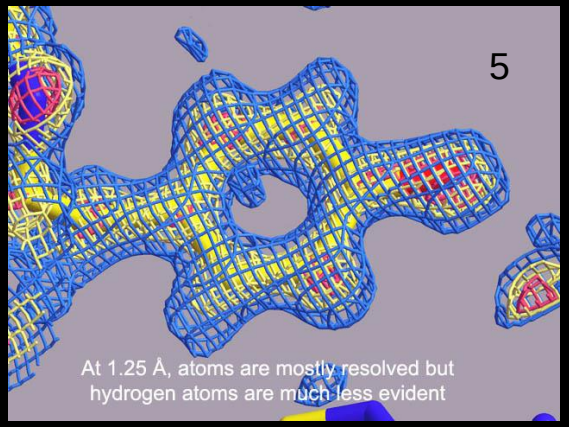
Pharmaceuticals work are designed and work at this level

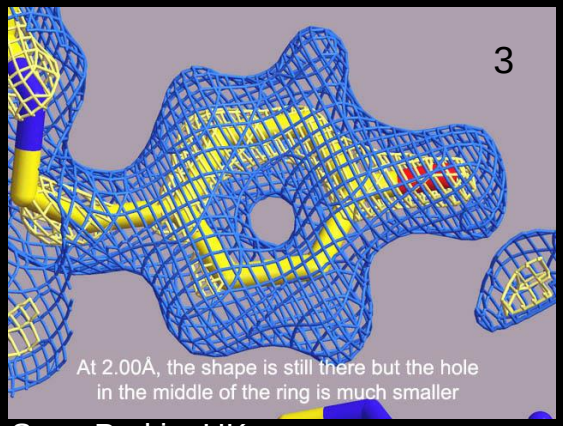
Biological machinery (proteins, DNA, RNA, viruses etc.) at this level is smaller then the wavelength of light – we cannot observe them with microscopes.

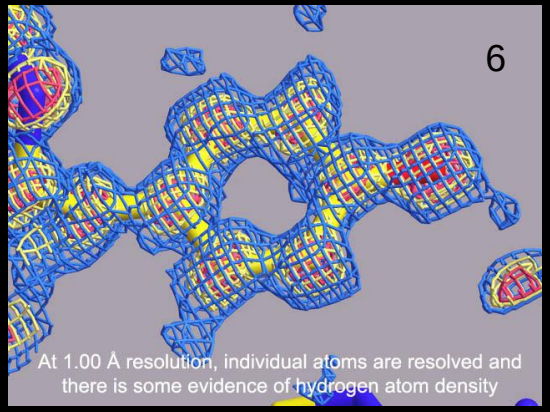












Sean Parkin. UK

Better quality crystals result in improved structural information (resolution).

Improved structural information aids in understanding of the mechanism.

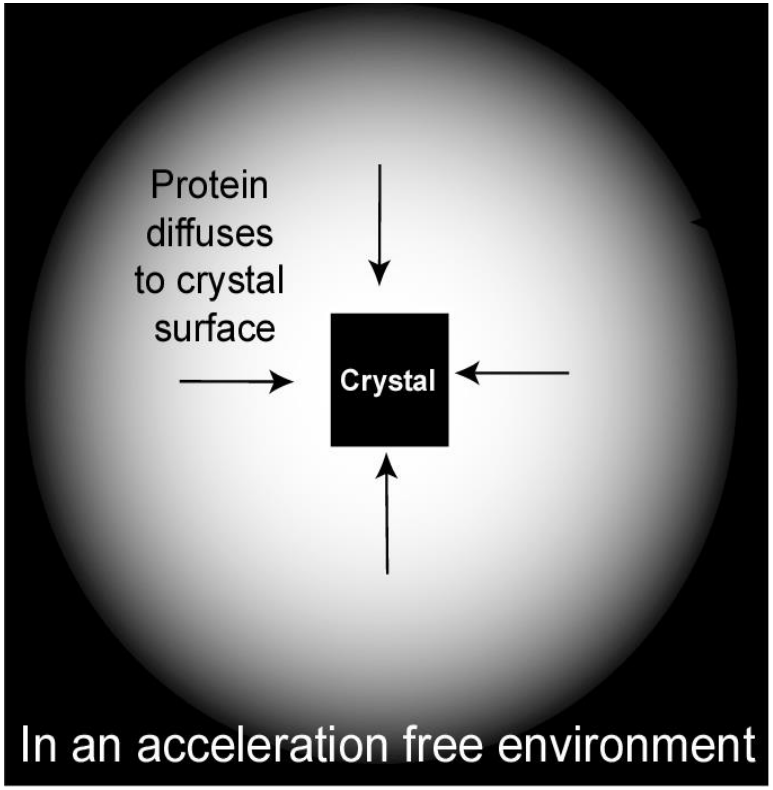
Understanding mechanism aids pharmaceutical design cutting years of development.

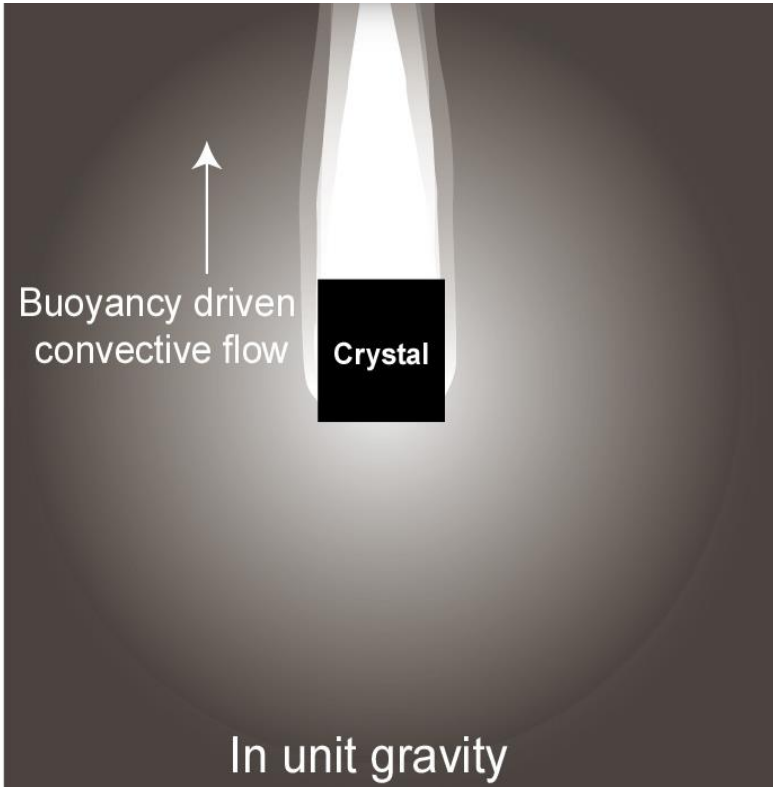
But microgravity does not directly enhance resolution….

- At the molecular length-scale reducing the effective gravitational forces will have no direct influence on short-range order.
    - Self assembly is unaffected. Sun et al., *Adv. Space Research* 24, 1341-1345, (1999) showed that coagulation of polystyrene spheres in a density matched liquid was not influenced by gravity for 0.1 μm particles and showed only a weak influence for 1.0 μm particles (a protein molecule is on the order of 0.01μm in dimension).
    - Brownian motion dominates. Prodi et al., *Atmospheric Research* 82, 379-384 (2006) showed that the displacement of particles in microgravity, due to Brownian motion, follows a Gaussian distribution like that at 1g.
    - Gravitational forces do not affect bond energies at the molecular level. Physical properties such as boiling and freezing points, enzyme kinetics etc. have not been observed to change, Giachetti et al., *Microgravity Sci. Technol*, 12, 36-40 (1999).
- Brownian motion dominates at the short-range level – that level that fundamentally determines the diffraction limit (amount of detail seen).
- Physically, for a well prepared sample microgravity growth has no direct effect on resolution.

So, why grow biological crystals in microgravity?

Microgravity conditions can be diffusion limited



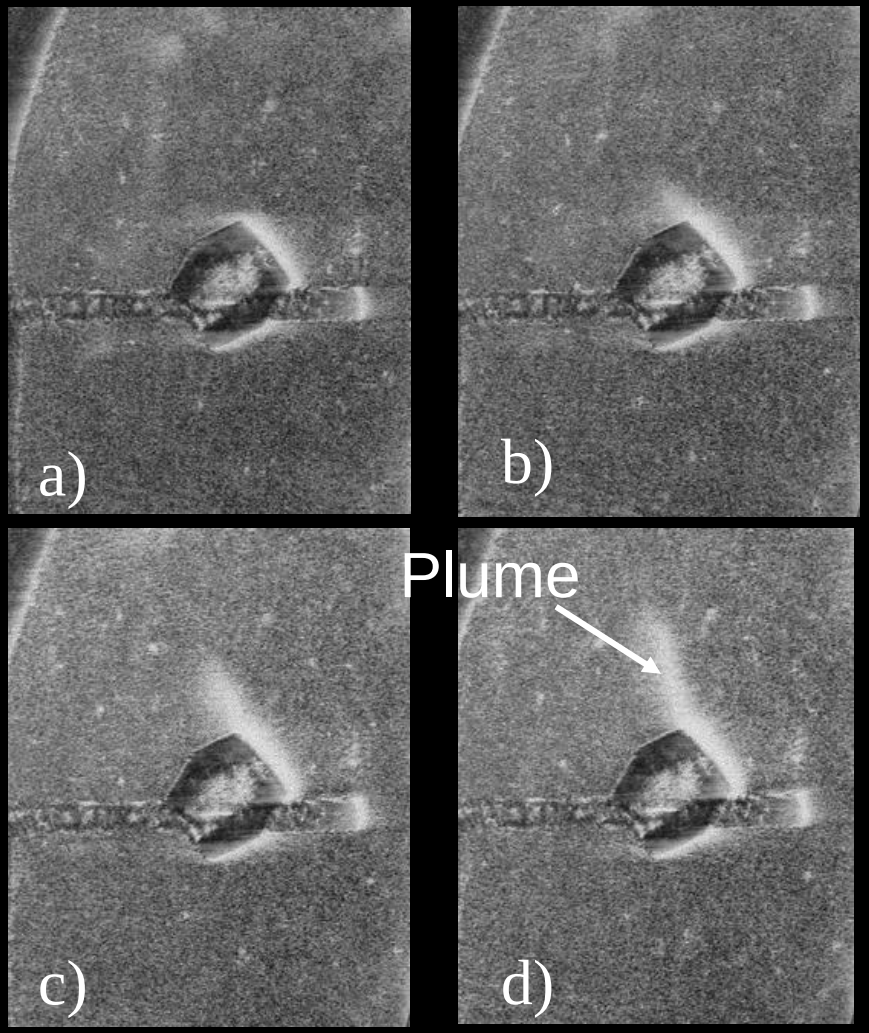


This can be observed experimentally (it's not a theory)

On the ground:

As the solution surrounding the crystal becomes depleted of the growing macromolecule the solution starts to rise due to density differences.

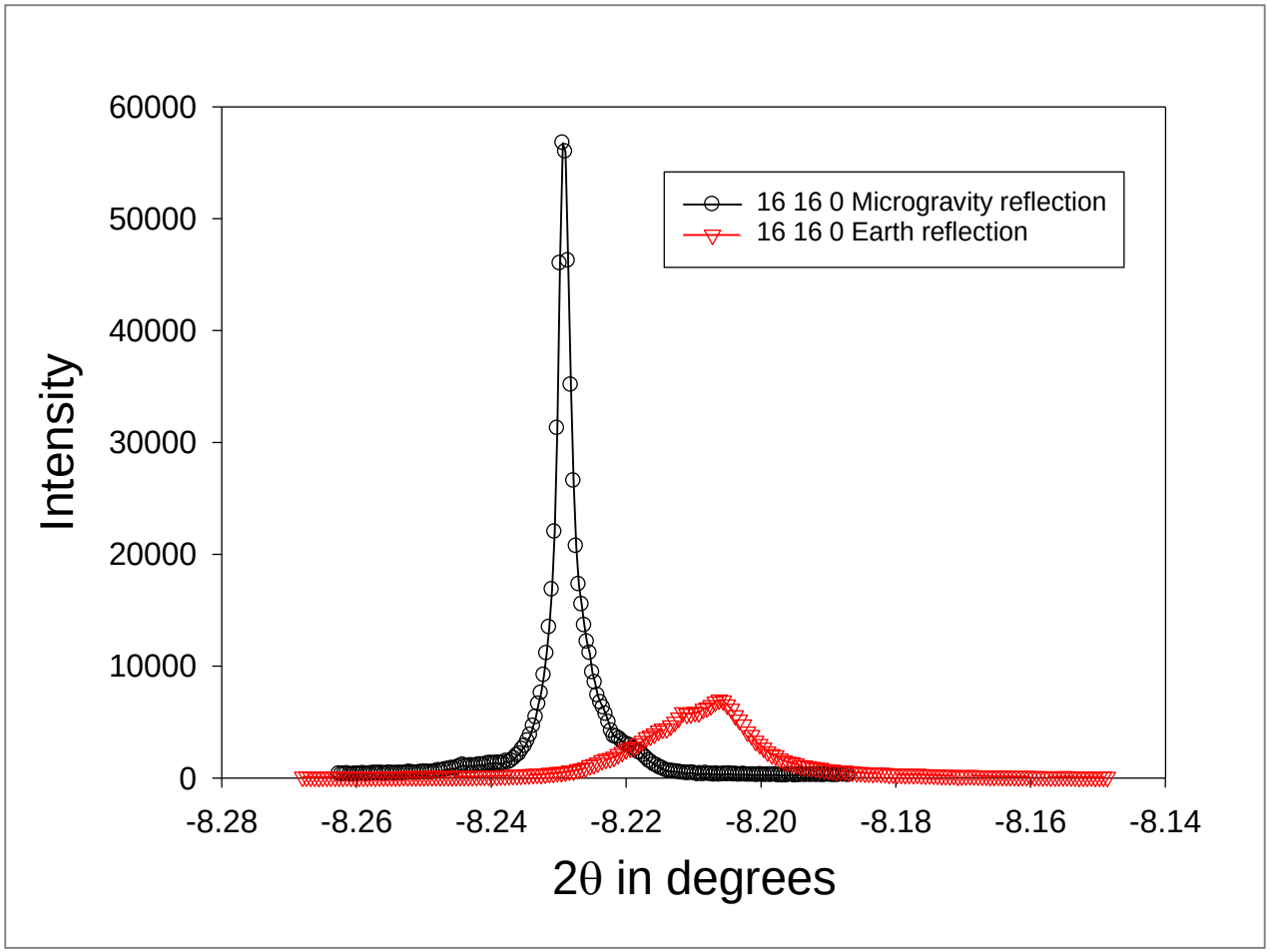
A convective growth plume of solution flows over the crystal face impeding growth and the quality of crystal packing.



Schlieren photograph of a growth plume rising from a lysozyme crystal (pH 4.0, 0.1M sodium acetate, 5% NaCl at 15°C.M.L. Pusey, J. Cryst. Growth, 122, 1-7, 1992) .

Long range order (length scales of many proteins in the crystal) can be improved.

Original experiments investigating microgravity crystal growth (mosaicity)



Identical reflections from microgravity and ground grown lysozyme.

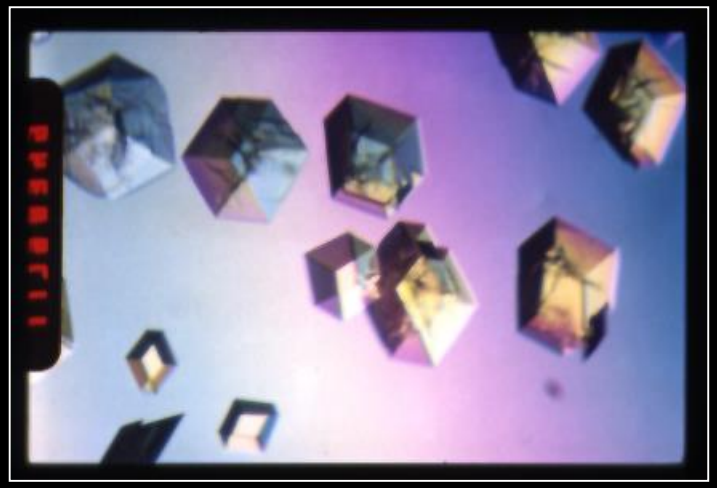
Eight times increase in signal to noise.

The larger illuminated volume only accounted for a doubling.

Microgravity 0.0023 degrees, ground 0.0130 degrees.

Snell et al., Acta Cryst. D51 099-1102 (1995

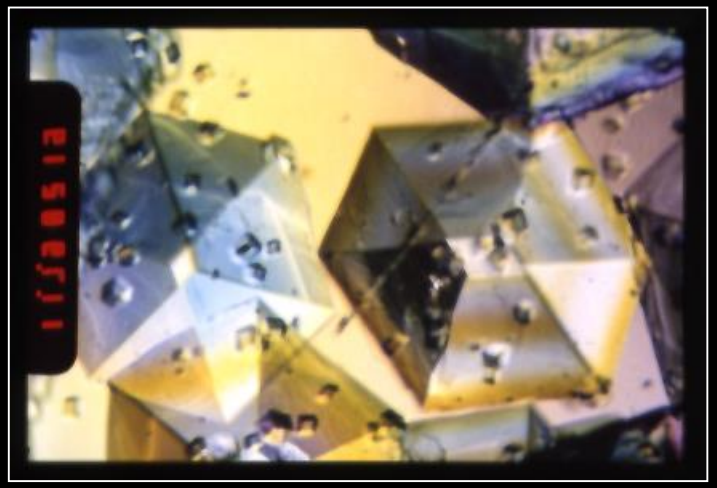
Previous studies on insulin



Images to same scale.

Ground:

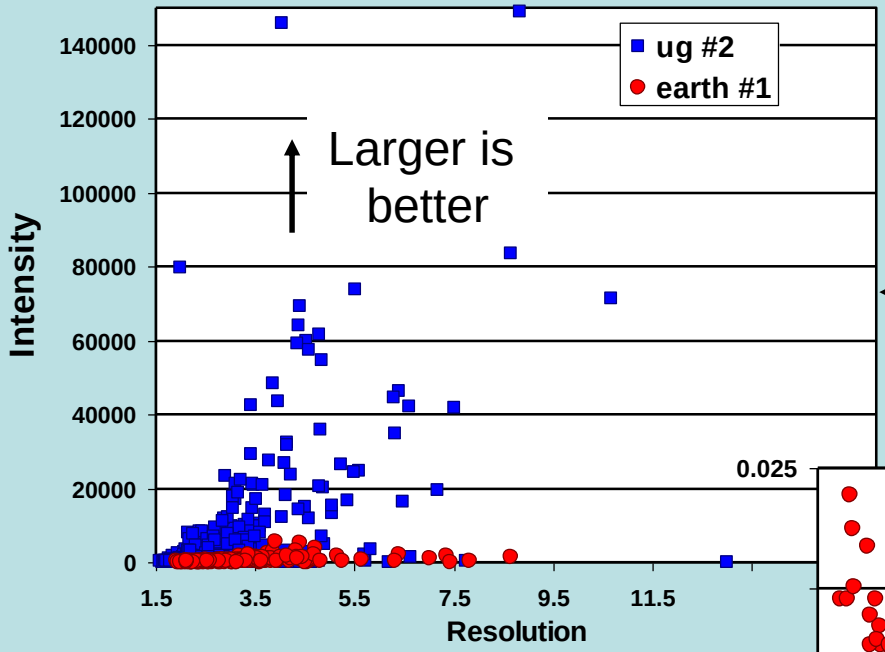
Sedimentation onto the bottom. Clumping of crystals.



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).



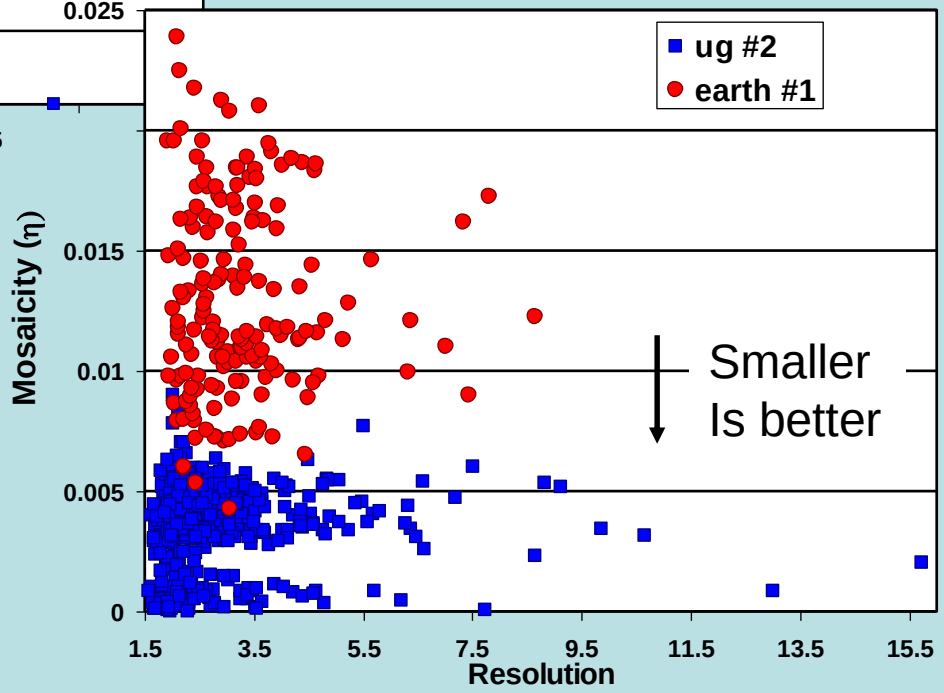
Microgravity Blue

Ground Red

Structural, short range improvement

Physical, long range improvement.

The worst microgravity and best ground crystal data are shown.



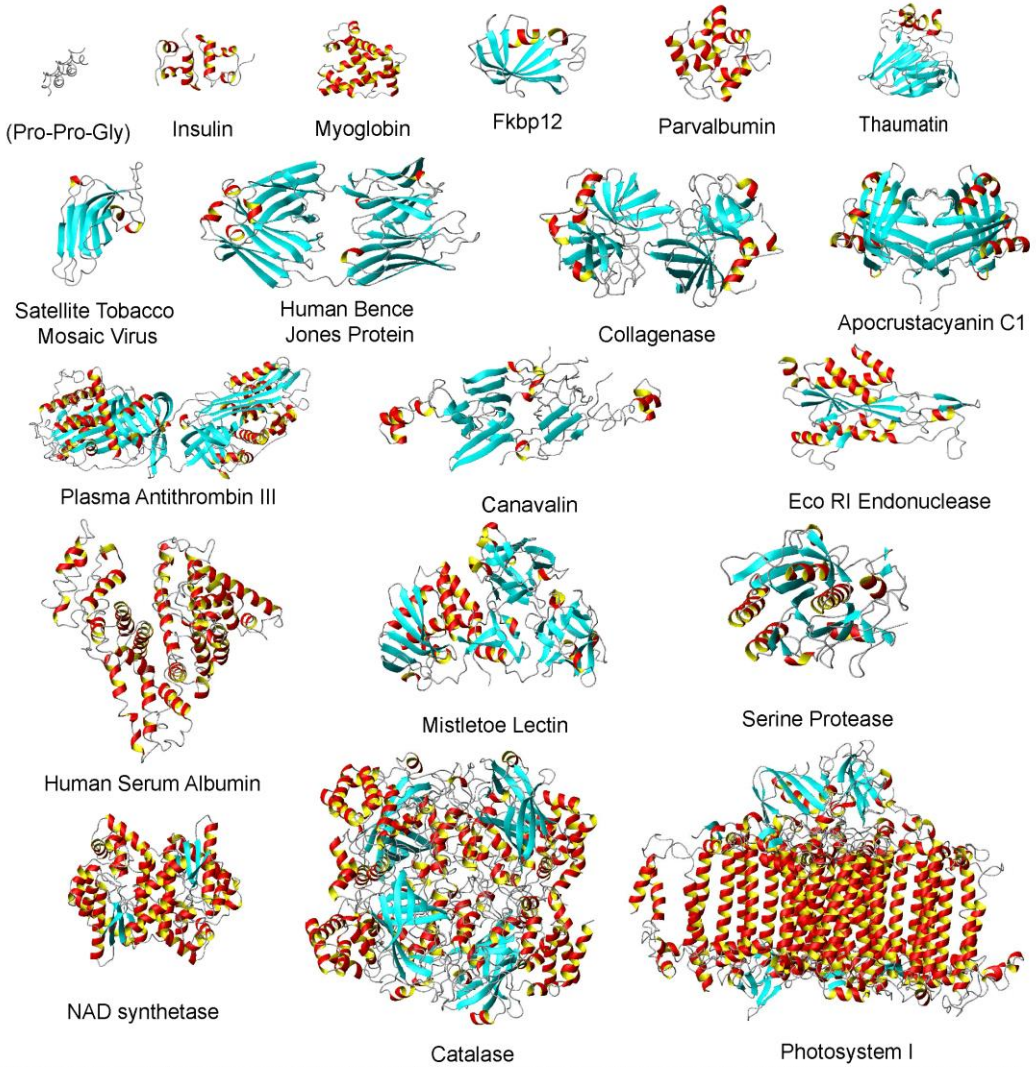
Effects of microgravity on crystallization

- Increase in volume (due to suspension in crystallization solution)
  - Leads to higher signal-to-noise, can be exploited to reduce radiation damage. Harder to cryocool (protect from radiation damage, 3Gy to kill a hamster, 30 Mgy used in crystallography)

- Reduction in mosaicity due to improved physical perfection.
  - Mosaicity reduced to a fraction of a degree. Each reflection extends over this angle and if you record in these angular steps you can optimize the signal. Destroyed by cryocooling.

Improved volume and reduced mosaicity result in improved structural knowledge

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.


Definitions
(what we are looking at)

Definitions:

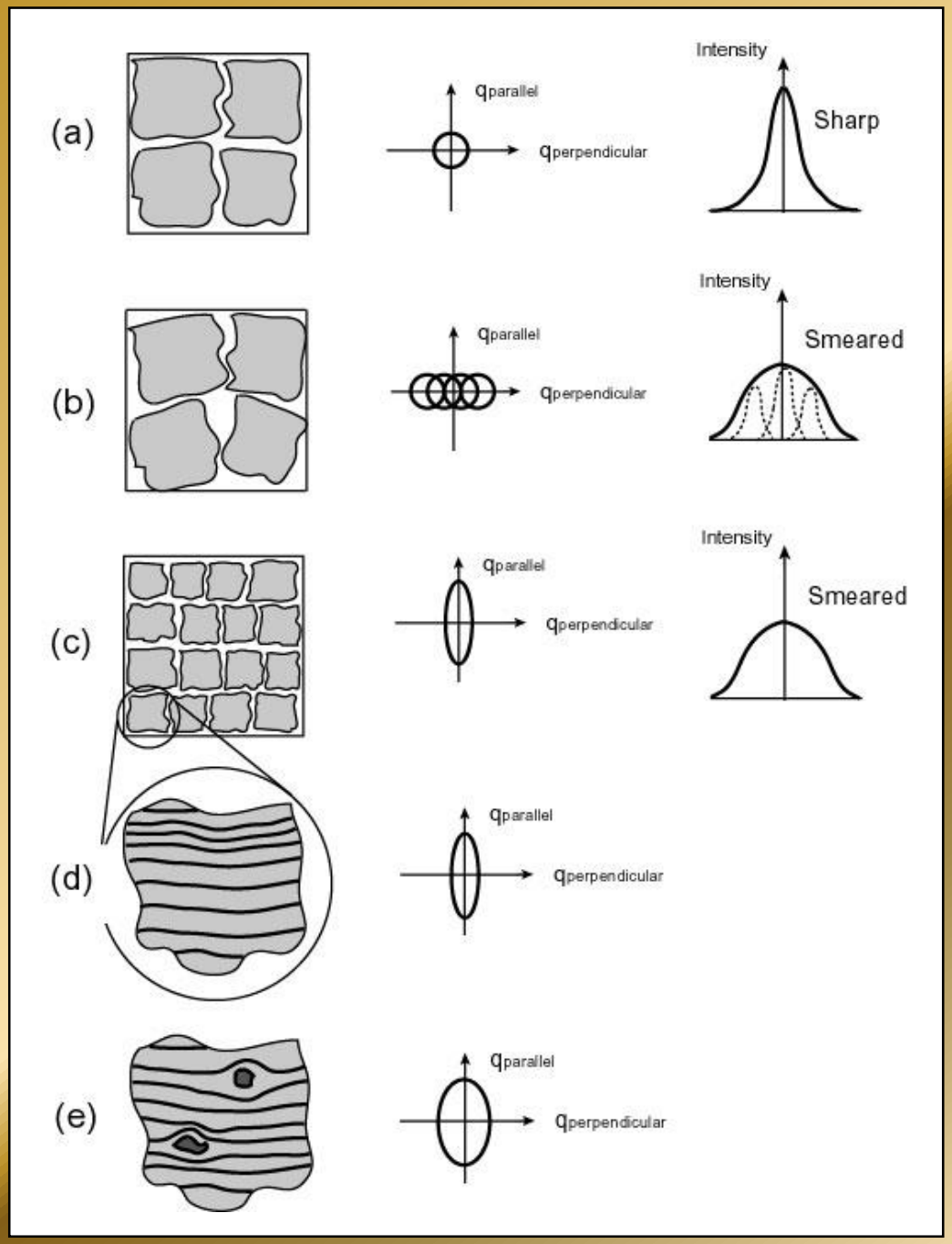
- Mosaicity
  - A crystal can be thought of as an array of domains all slightly misaligned with each other. Perturbations to the misalignment are lumped together in the quantity called mosaicity. A highly mosaic crystal has many imperfections.
- Growth rate dispersion
  - Individual crystals of the same size, all apparently subjected to identical growth conditions, can grow at different growth rates.
- Quality
  - A high quality crystal has low mosaicity and diffracts strongly to a high resolution.

More on Mosaicity

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*.

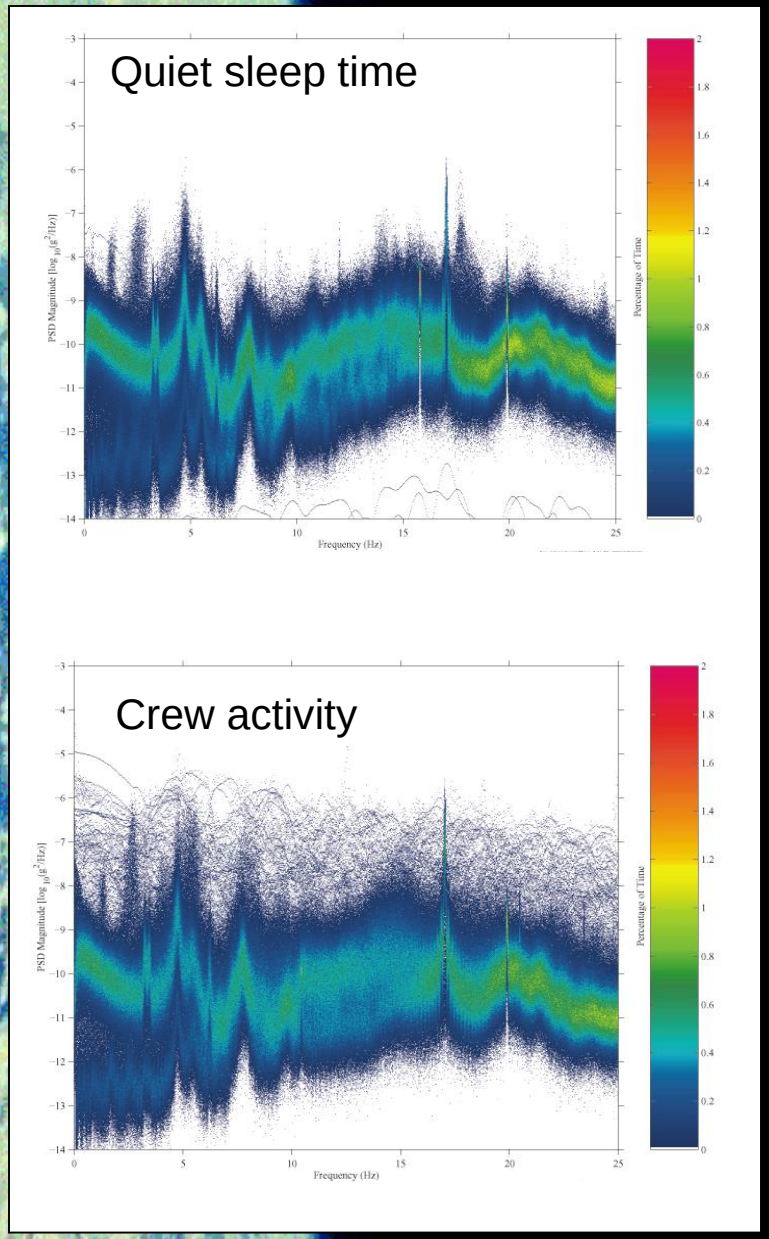
Misalignment, volume and unit cell variation all have effects in reciprocal space (smearing out reflections) which can be represented by the Darwin model.

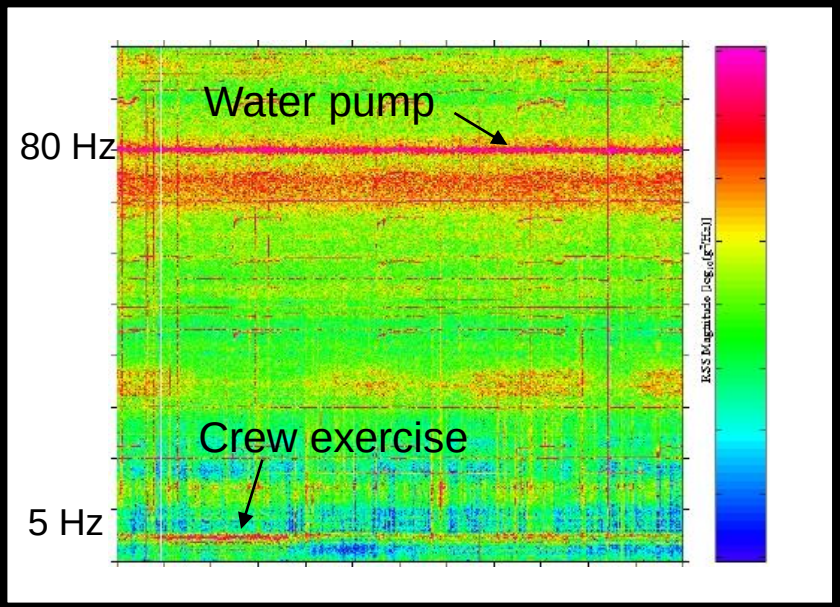


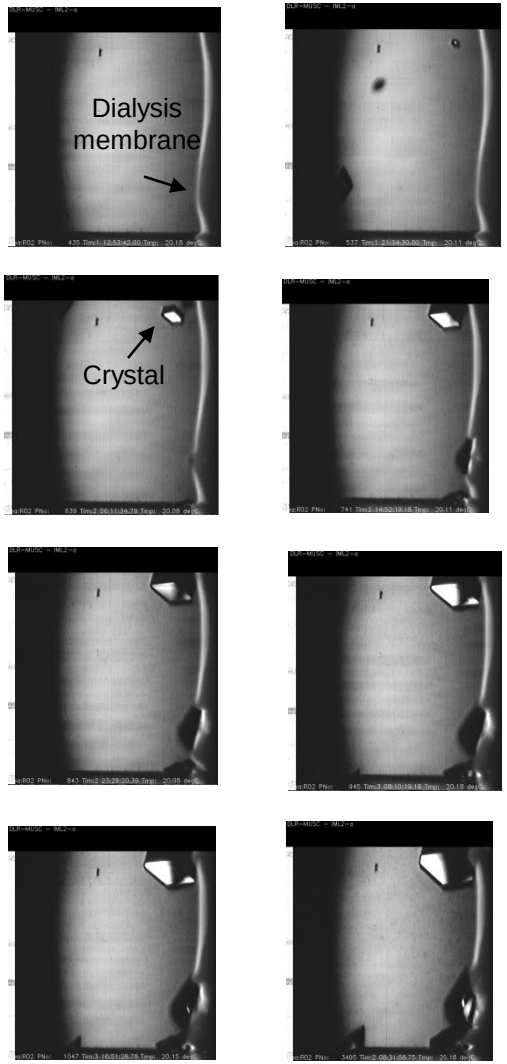
Can we observe the effect of changing acceleration levels on growth?

Does acceleration affect growth?

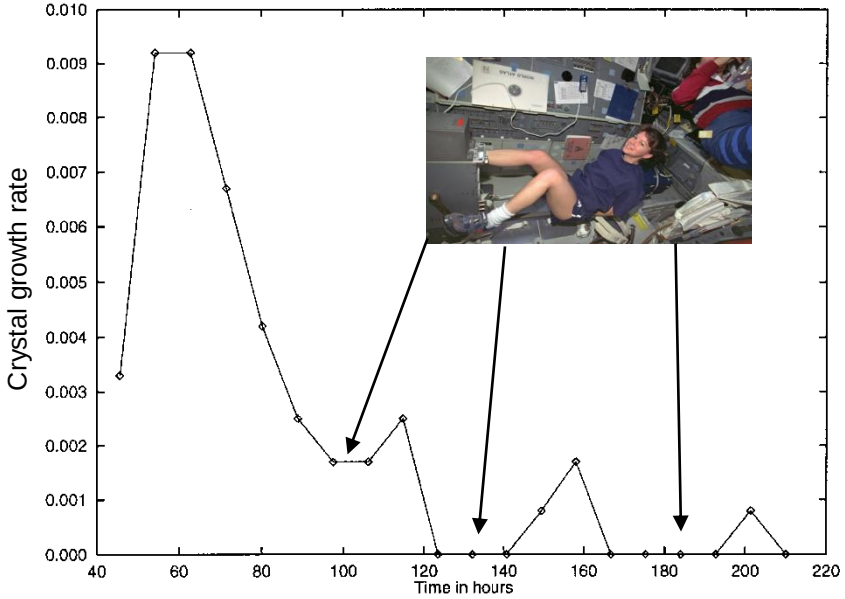
- The microgravity acceleration environment is noisy.
- This noise can be seen in the crystal growth experiments.
- We do not want this noise to mask our growth rate experiments.
- We need to measure the noise during the experiment to correlate it with any unexpected observations

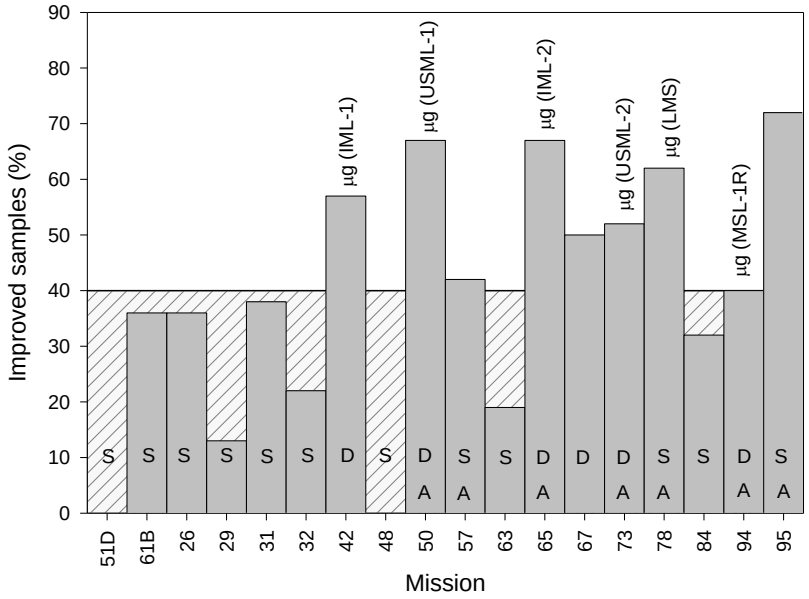


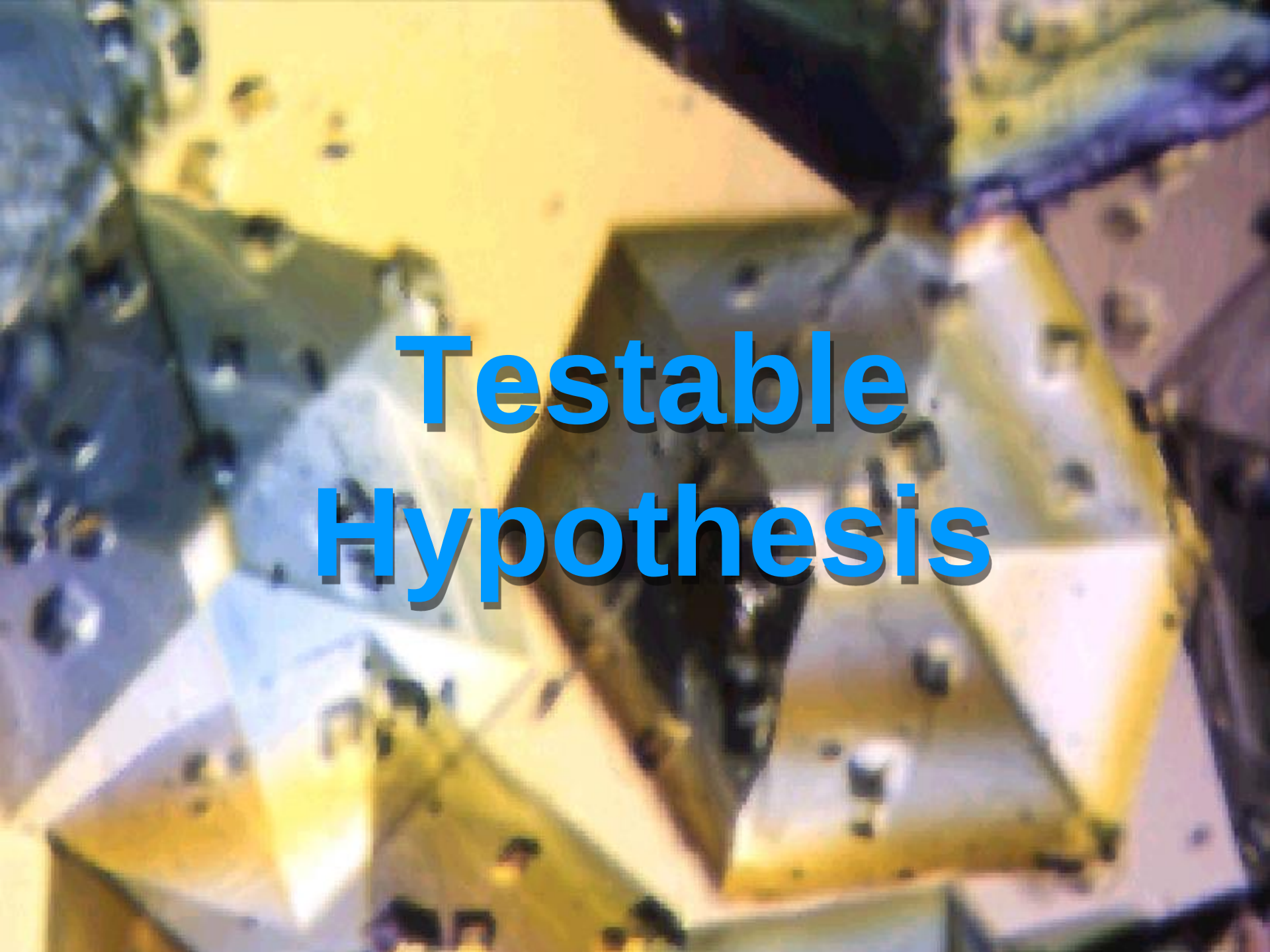




Single crystal imaged over time on STS 65. Snell at al. (1997)



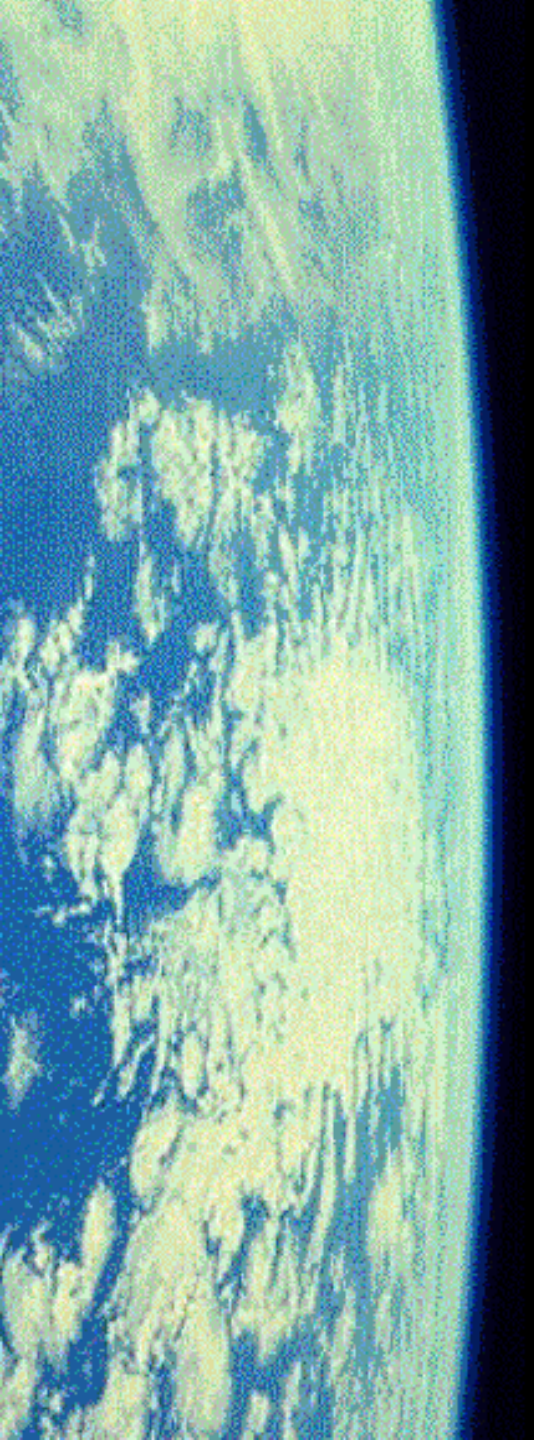



Testable
Hypothesis

Relationship between growth rate dispersion and mosaicity

Small molecule studies have shown a direct relationship between mosaicity and growth rate dispersion.

- Sherwood and Ristic (2001) see reduced mosaicity with reduced growth rate dispersion for sodium chlorate, potash alum and sodium nitrate.
- The same effect is also seen for sodium chloride (Cunningham *et al*., 1991) and ammonium sulfate (Meadhra *et al*., 1995).
- Larger molecules such as sucrose (Berglund *et al*., 1984) and fructose (Johns *et al*., 1990) show dispersion.
- Growth rate dispersion studies for macromolecules have been limited. Ovalbumin (Judge *et al*., 1995) and lysozyme (Cherdrungsi, 1999) are two example cases.

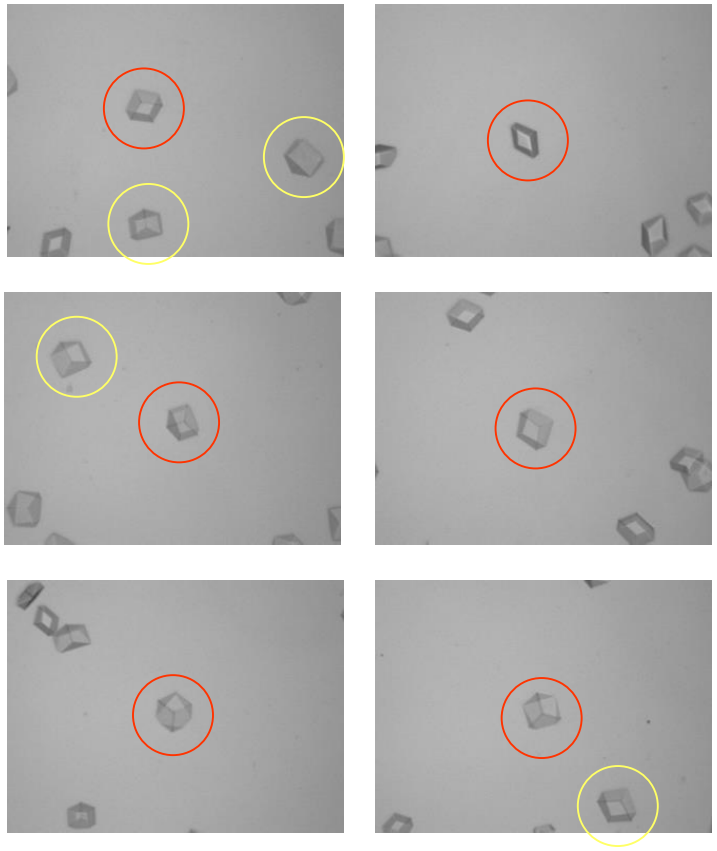


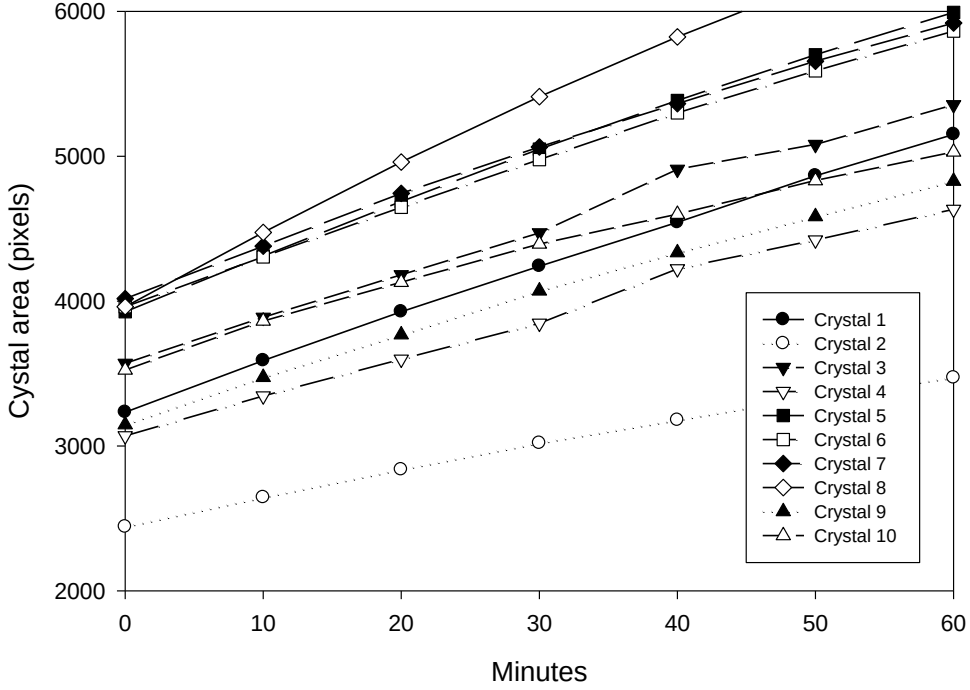
- In small molecule studies X-ray data shows that growth rate dispersion is related to crystal mosaicity. The greater the growth rate dispersion the greater the mosaicity.
- Mosaicity dramatically improves in microgravity grown crystals.
- Microgravity crystals with reduced mosaicity can be used to increase the data signal-to-noise and hence resolution.

Hypothesis

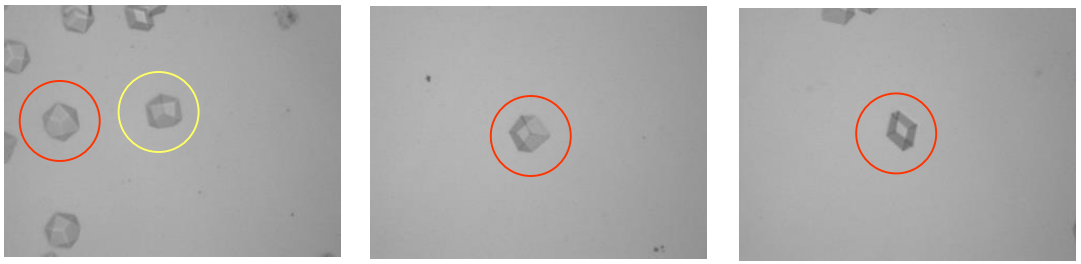
- Growth rate dispersion is a predictive experimental technique for improvement in microgravity. Crystals benefiting most from microgravity will be those that show most growth rate dispersion on the ground.

More on Growth Rate Dispersion

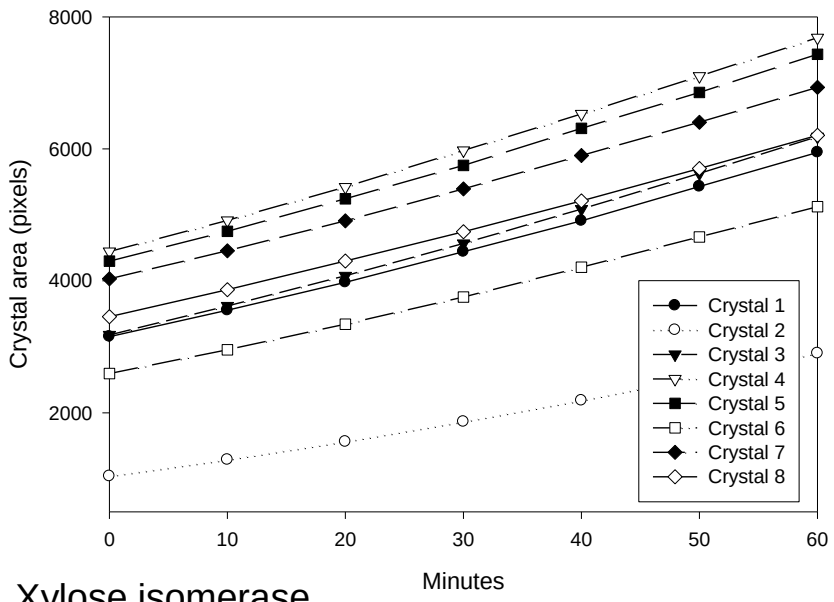




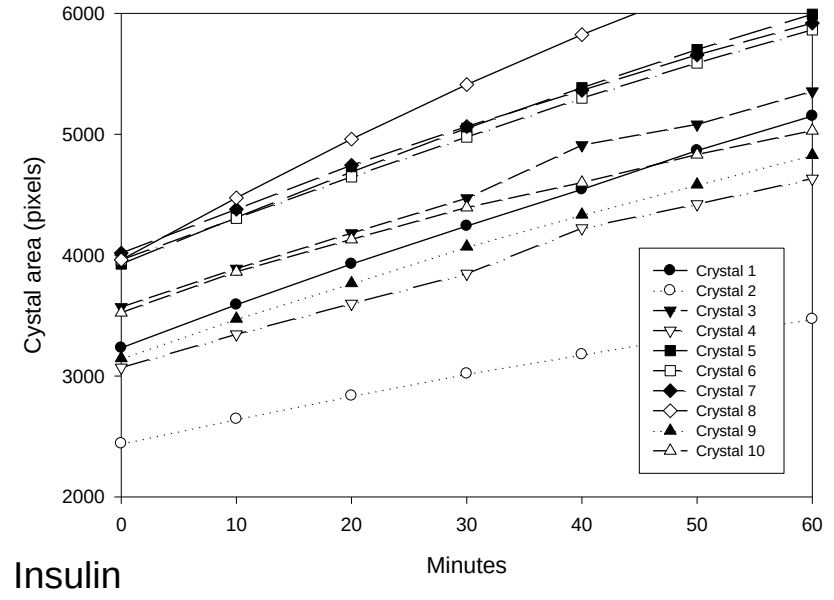
Nine out of 10 imaged insulin crystal samples shown outlined in red. Crystals outlined in yellow are also useable for data.



Crystals under seemingly identical biochemical conditions grow at different rates. Growth rate dispersion is a measure of the differences in rates.



Xylose isomerase



Insulin

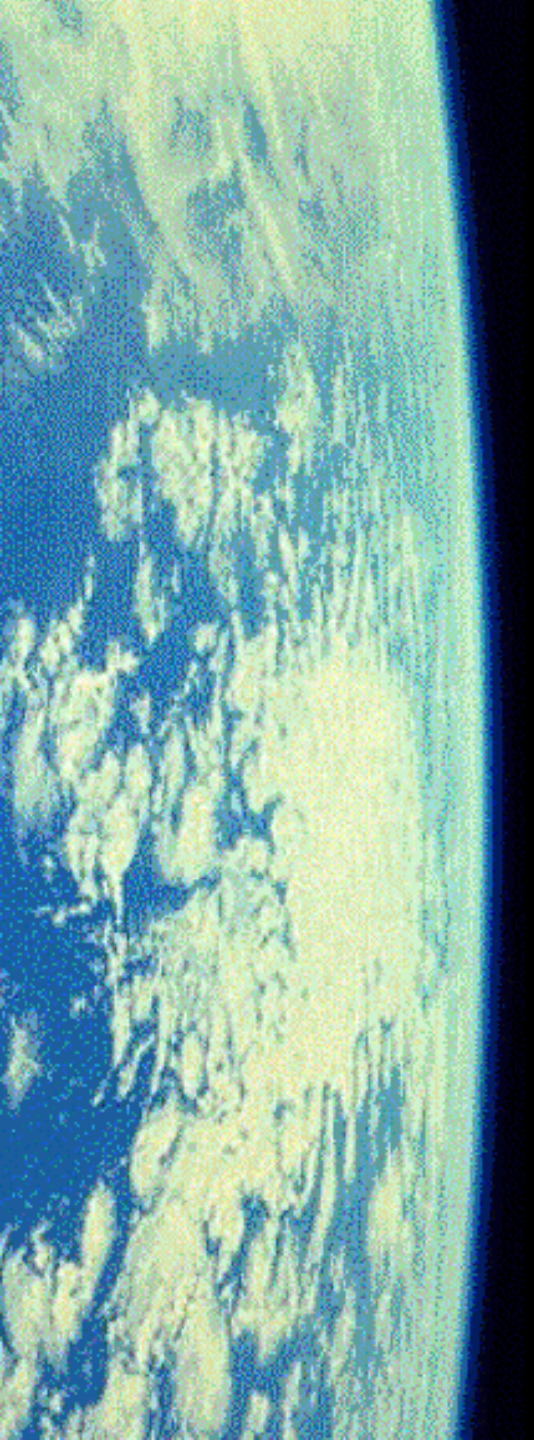
Current results

In each graph the first 60 minutes of observations are fitted with a linear function and the gradient, or growth rate, calculated.

The growth spread coefficient (growth rate dispersion) is obtained by dividing the standard deviation of growth rate by the average growth rate.

For insulin we get a growth spread coefficient of 0.28 and for xylose isomerase a growth spread coefficient of 0.14.

Insulin has shown improvement in microgravity, xylose isomerase has not.

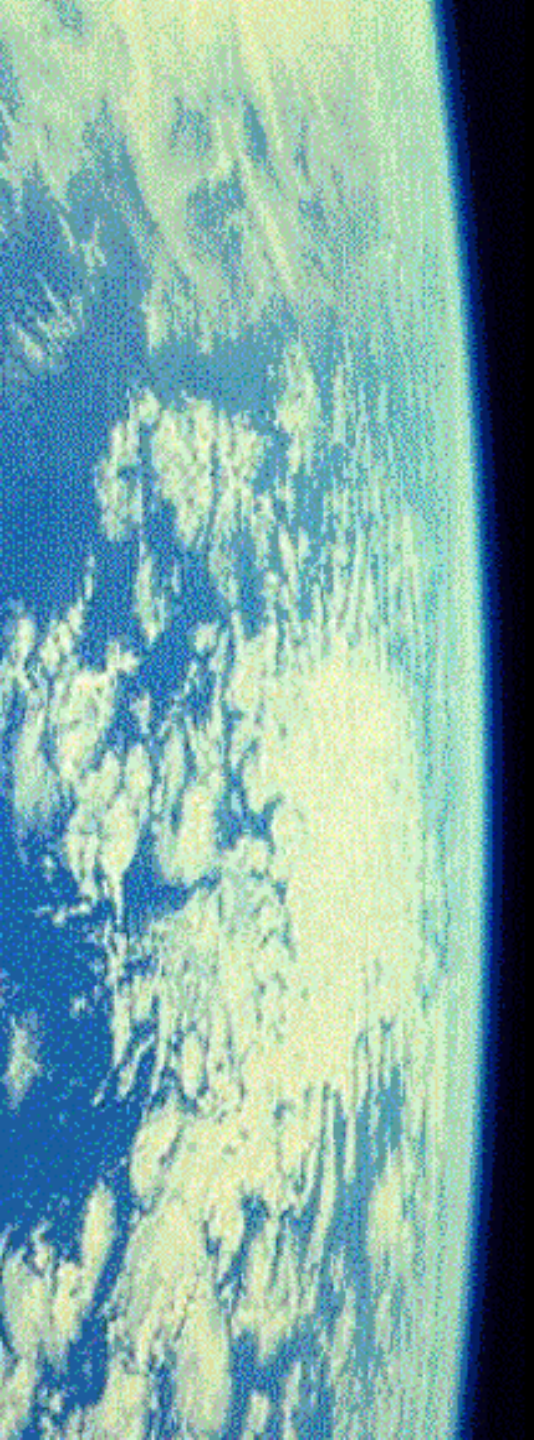


Our Aims

1. Take a single protein and selectively mutate the surface residues to impact growth and growth rate dispersion.
2. Monitor the crystal growth over time on the ground selecting those that show both large and small growth rate dispersion and analyze them by X-rays on the return to earth.
3. Test the hypothesis and if it is valid (which all the preliminary data indicates) measure growth rate dispersion on the ground for a set of health related proteins and make predictions of resultant quality from growth on orbit.
4. Use growth rate dispersion as a filter for samples likely to benefit.

Use the best facilities in space with the best on the earth

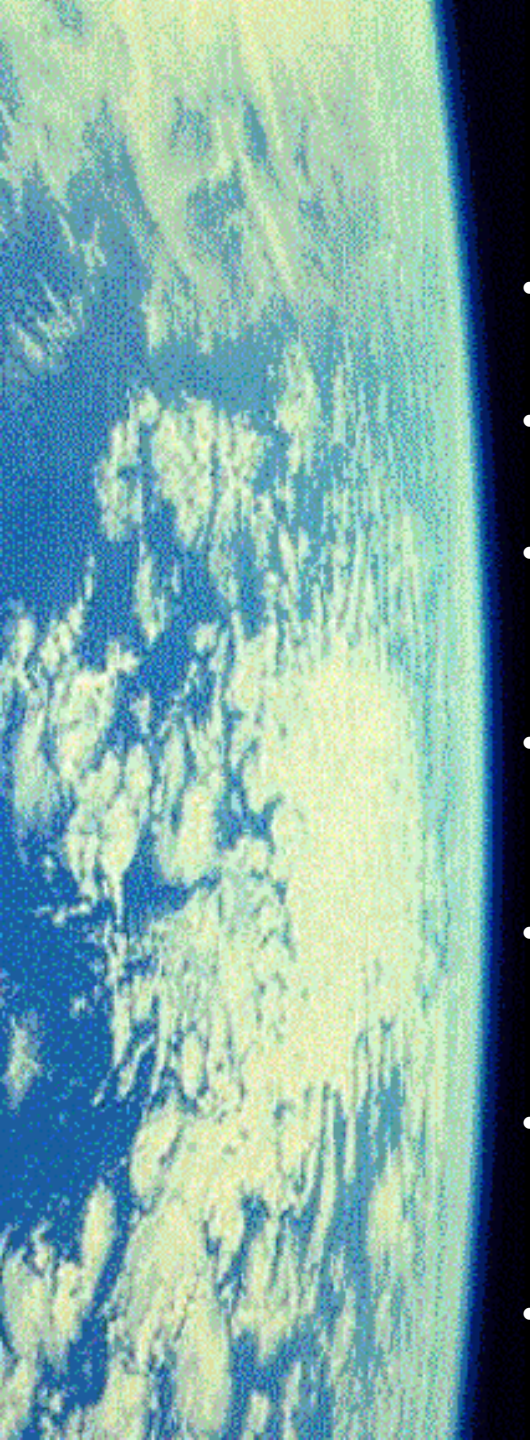

So What?
(what do we get and
what does it mean)



What do we get?

- We get a technique that predicts success in microgravity based on a simple experiment on the ground.
- This means we can save experimental slots for samples that we know will improve.
- Every sample flown will have a much greater chance of providing more structural data and advancing our knowledge.
- We’ll be playing the game with loaded dice.


Summary
(what to take away from this)



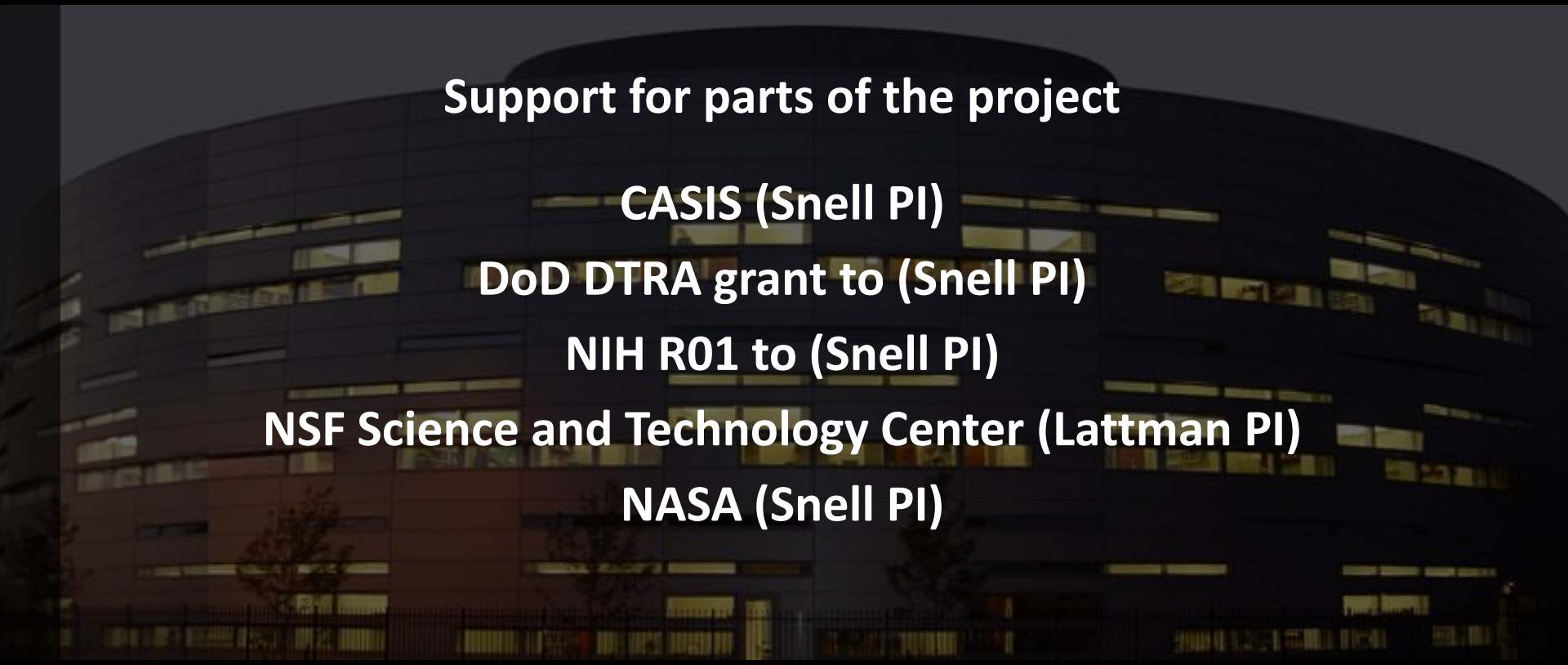
Summary

- Based on previous experimental data we see clear improvement of mosaicity in microgravity.
- Studies with several small molecule samples show that mosaicity is related to growth rate dispersion.
- Our data shows that growth rate dispersion is large for a sample that clearly improves in microgravity and small for one that does not.
- Our hypothesis is that growth rate dispersion on the ground is a predictive technique of a sample that will improve from flight.
- Our results will provide a predictive technique - We can improve the use of microgravity with simple experiments on the ground.
- Our results will also point to a more fundamental understanding of just how microgravity is improving crystal quality.
- Hopefully, a small experiment will have a big impact.

BioXFEL

Eight post-doctoral positions available at Buffalo, Phoenix, Cornell, Stanford and Milwaukee in the US plus one at Hamburg

Leveraging Support



Support for parts of the project

CASIS (Snell PI)

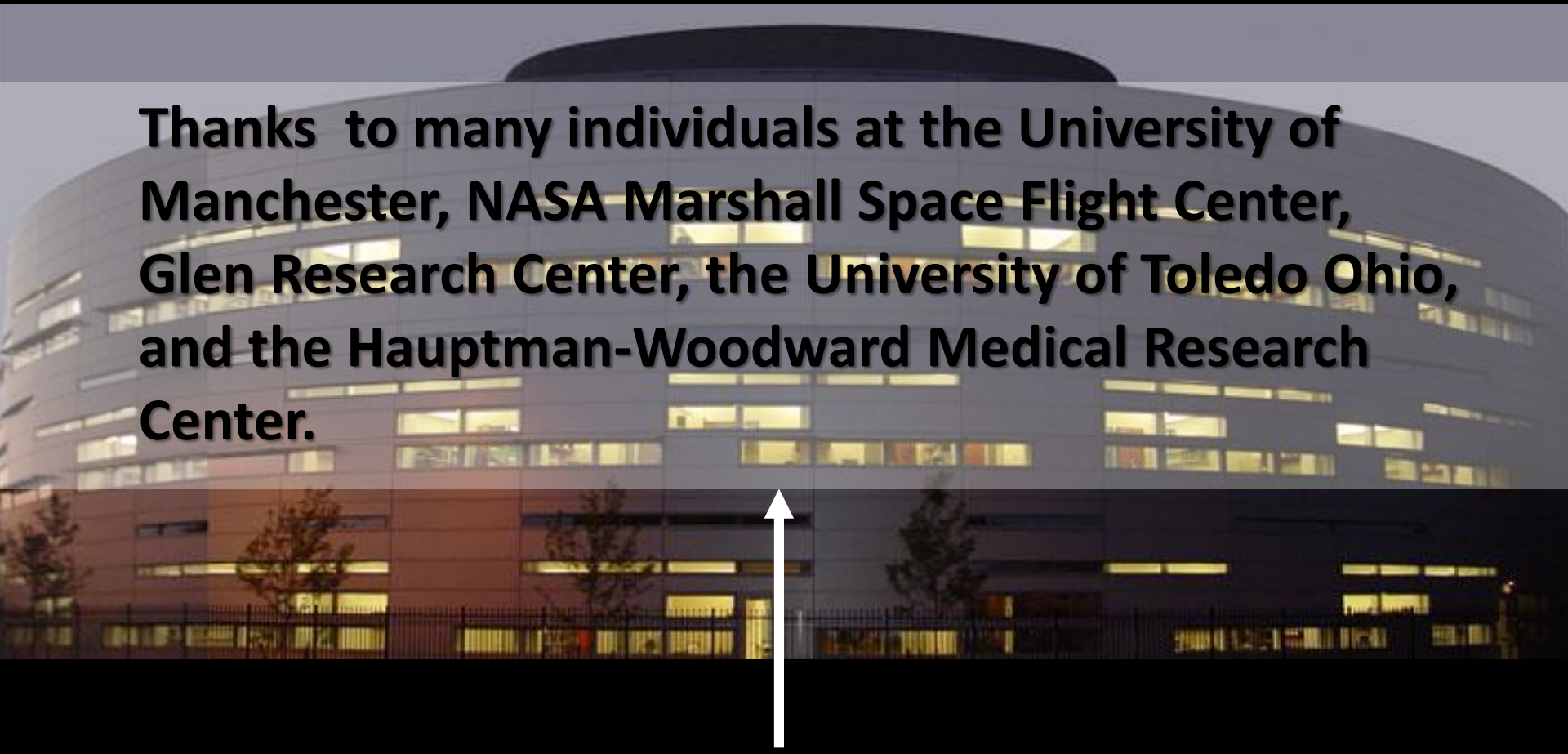
DoD DTRA grant to (Snell PI)

NIH R01 to (Snell PI)

NSF Science and Technology Center (Lattman PI)

NASA (Snell PI)

Thank you and questions?



Thanks to many individuals at the University of Manchester, NASA Marshall Space Flight Center, Glen Research Center, the University of Toledo Ohio, and the Hauptman-Woodward Medical Research Center.

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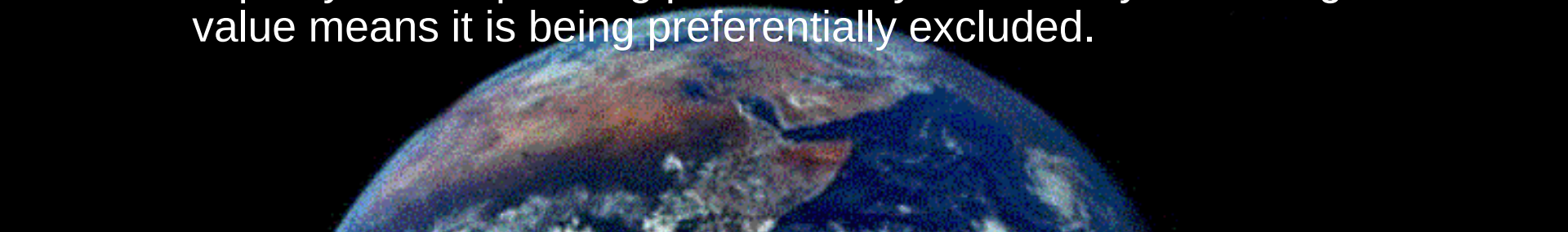
Impurity partitioning calculation

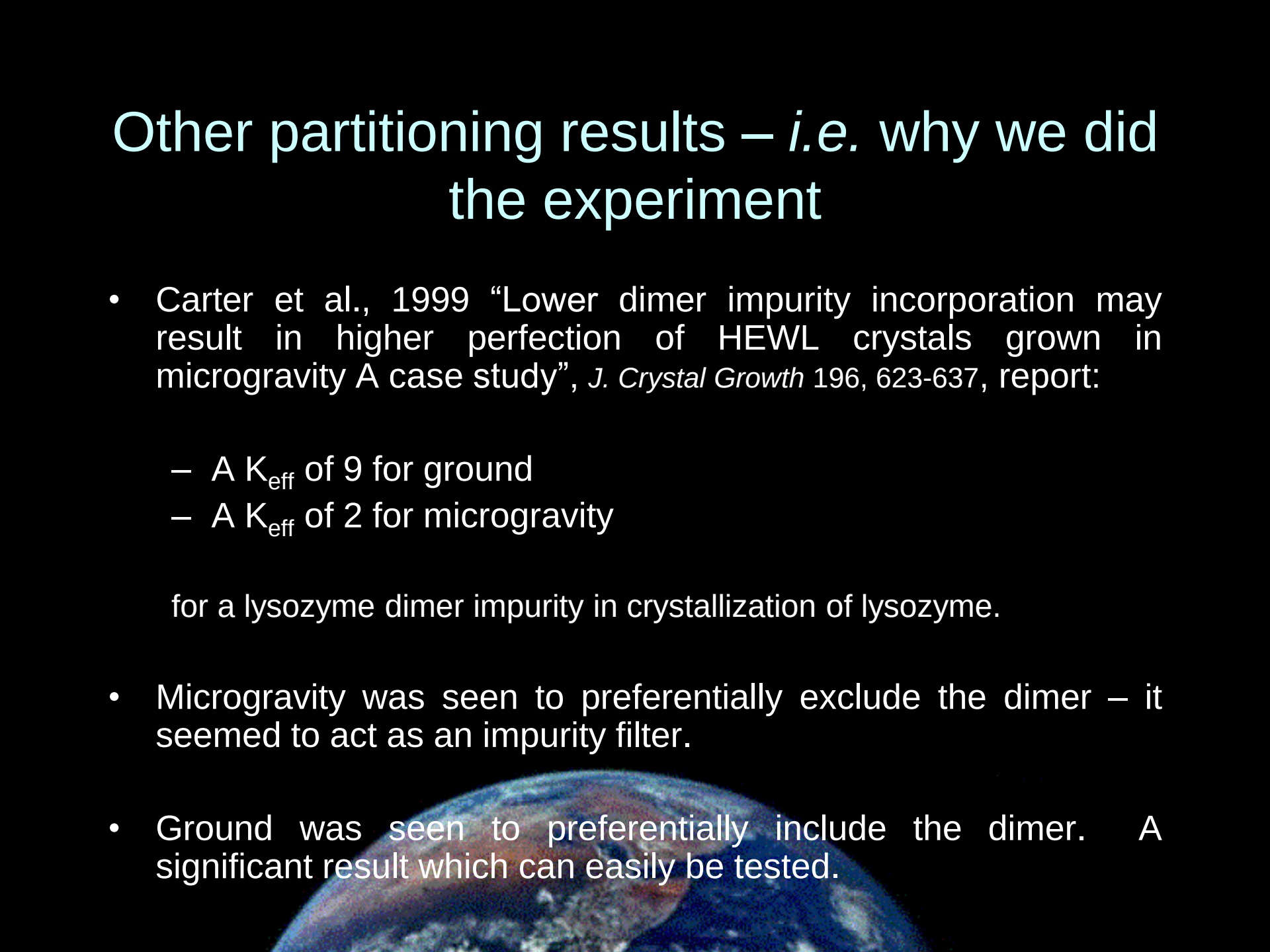
- Impurity partitioning was calculated according to Carter et al., 1999 J. Cryst. Growth 196, 623-627;
  - $K_{eff} = (C_{iS}/C_{pS})/(C_{iL}/C_{pL})$
- Where C_{iS}, is the concentration of impurity in the solid crystal, C_{pS}, is the concentration of the major protein in the crystal, C_{iL}, is the concentration of impurity in the initial solution and $C_{pL,}$ is the concentration of the major protein in the initial solution.
- What does it mean – A positive value of K_{eff} means that the impurity is incorporating preferentially into the crystal, a negative value means it is being preferentially excluded.



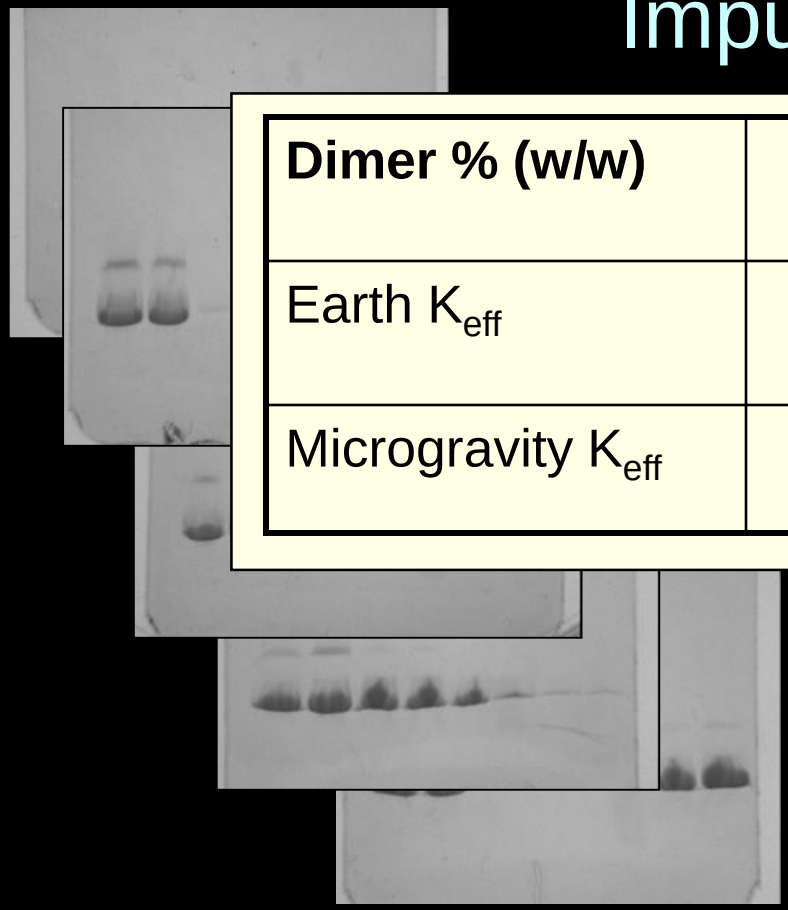
Other partitioning results – *i.e.* why we did the experiment

- Carter et al., 1999 “Lower dimer impurity incorporation may result in higher perfection of HEWL crystals grown in microgravity A case study”, *J. Crystal Growth* 196, 623-637, report:
  - A K_{eff} of 9 for ground
  - A K_{eff} of 2 for microgravity

  for a lysozyme dimer impurity in crystallization of lysozyme.
- Microgravity was seen to preferentially exclude the dimer – it seemed to act as an impurity filter.
- Ground was seen to preferentially include the dimer. A significant result which can easily be tested.



Impurity partitioning



Dimer % (w/w)	3.6	1.8	0.9	0.5
Earth K_{eff}	1.0	1.0	0.7	0.3
Microgravity K_{eff}	0.7	0.6	1.2	2.0

- Within the bounds of error there is no difference in partitioning for 3.6, 1.8 and 0.9% impurities.
- Microgravity preferentially incorporates the dimer at 0.5%

Snell et al., Investigating the Effect of Impurities on Macromolecule Crystal Growth in Microgravity, *Crystal Growth and Design*, 1, 151-158, (2001)