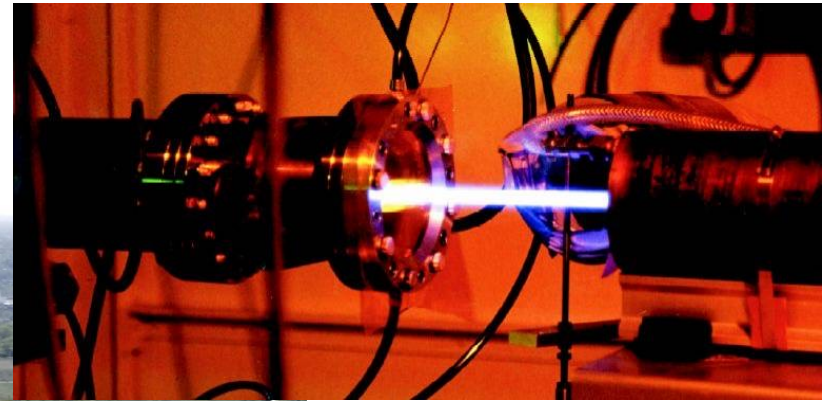


Coherent X-ray Diffraction Methods in Biology

- Ian Robinson
 - Sébastien Boutet
 - Franz Pfeiffer
 - Ivan Vartanians
 - Ross Harder
 - Meng Liang
 - Garth Williams
 - Mark Pfeifer
 - Jing Tao
 - Jim Zuo
- London Centre for Nanotechnology,
University College London
Diamond Light Source
(ex University of Illinois)
- Astbury Seminar
Leeds University
October 11th 2007

Synchrotron Radiation

Urbana



34-ID-C



Diamond Light Source (RAL)



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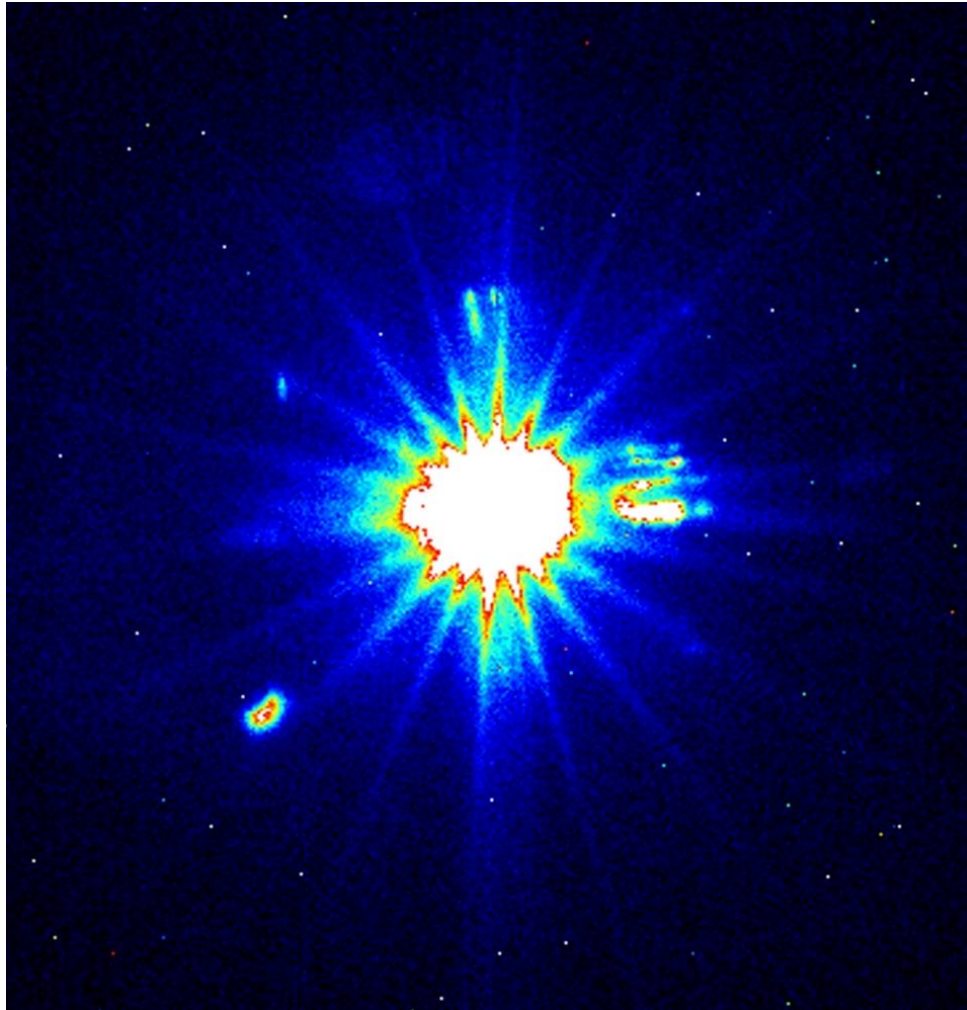
Last Girder – March 2006



I. K. Robinson Astbury 2007

“First Light” - May 2006

ramped to 3GeV – Sept 2006



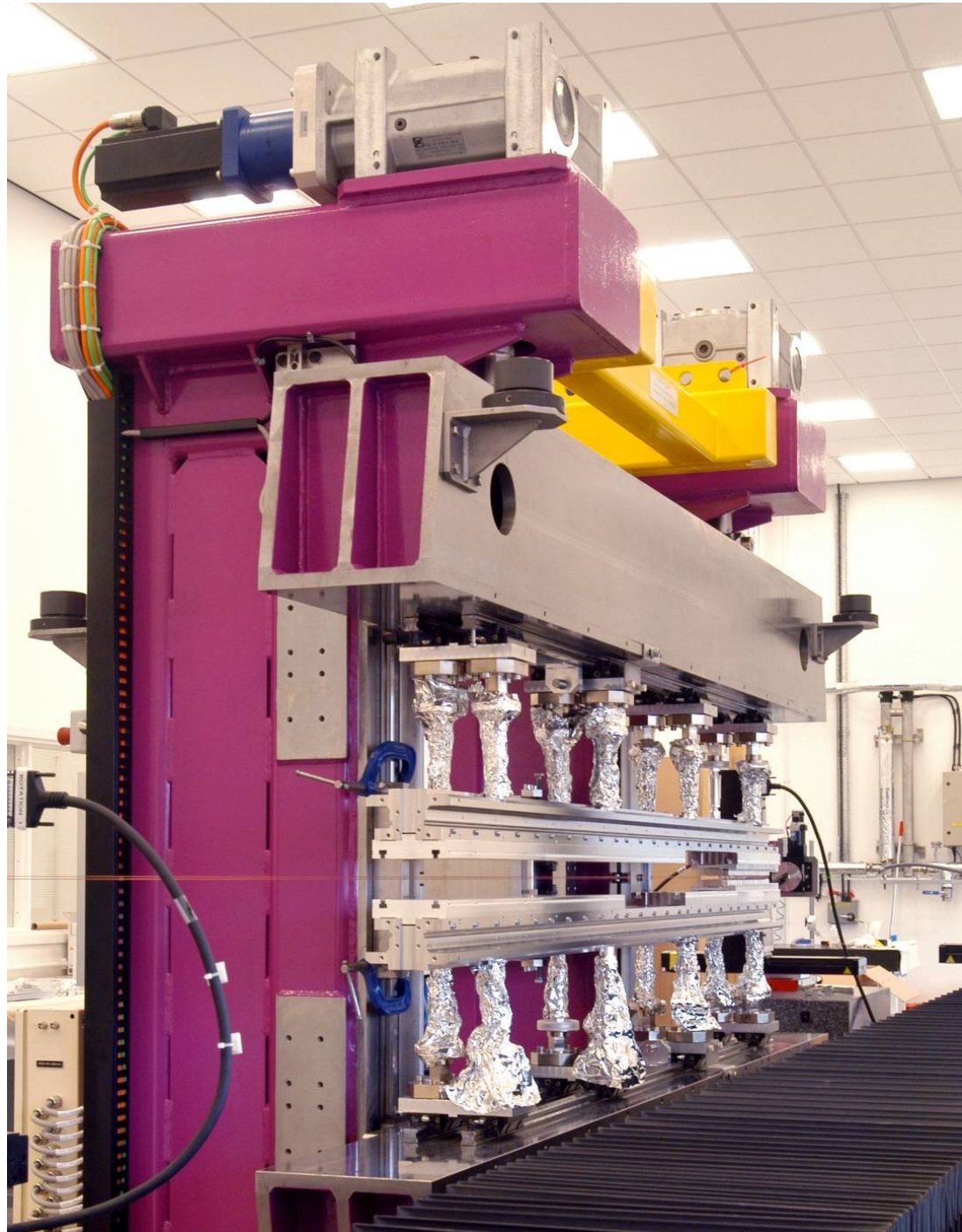
I. K. Robinson Astbury 2007

Typical beamline: I-22

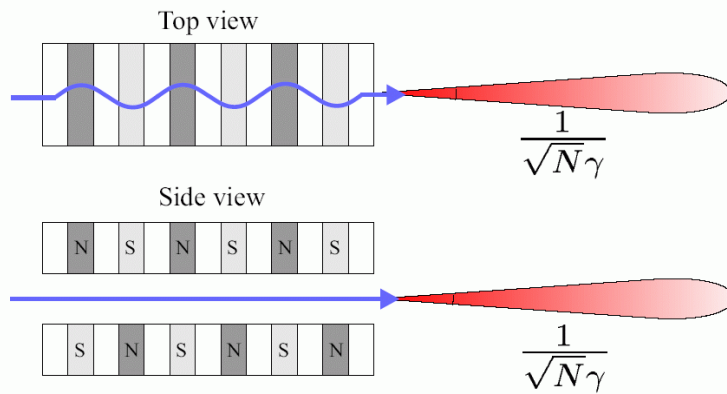


I. K. Robinson Astbury 2007

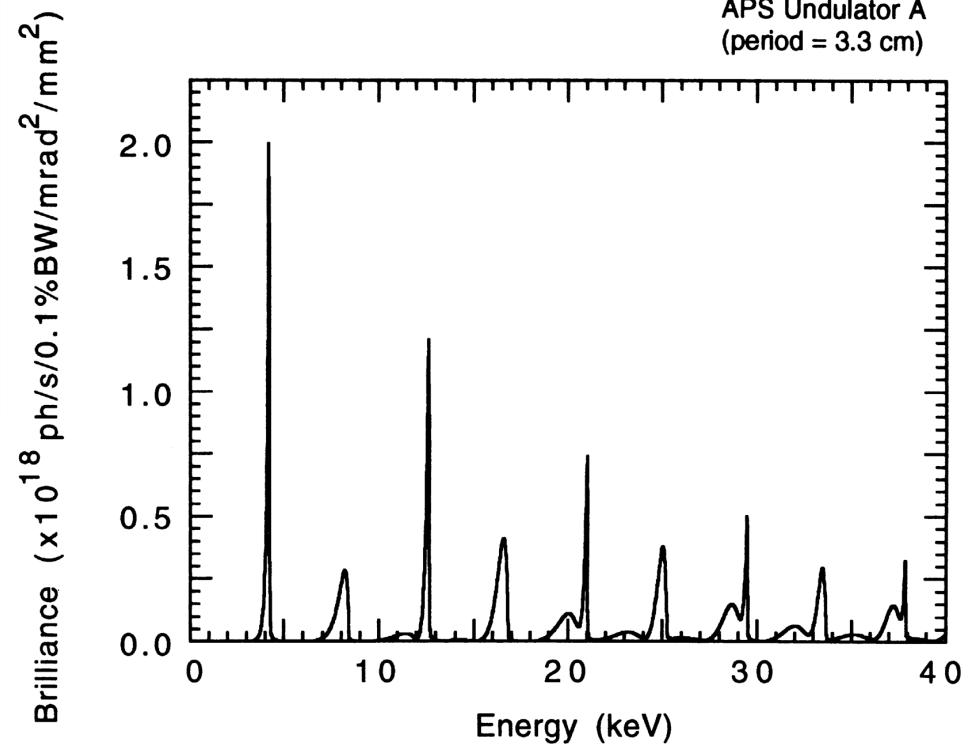
Diamond
in-vacuum
X-ray
Undulator



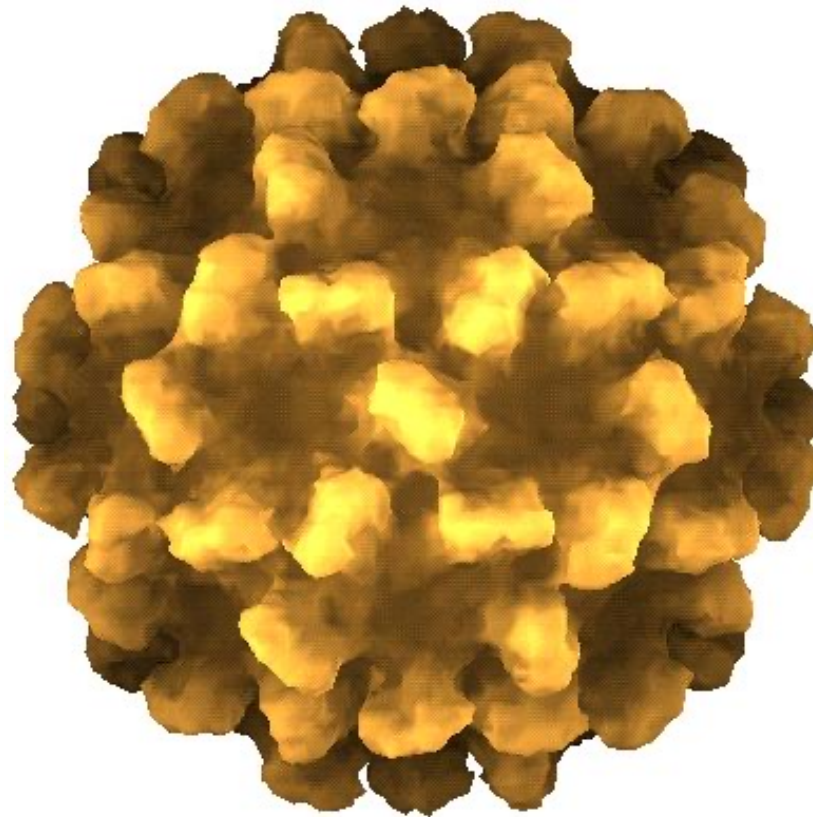
X-ray Undulator Principle



$$\lambda_X = \frac{\lambda_U}{2\gamma^2} \left\{ 1 + \frac{K^2}{2} + (\gamma\theta)^2 \right\}$$



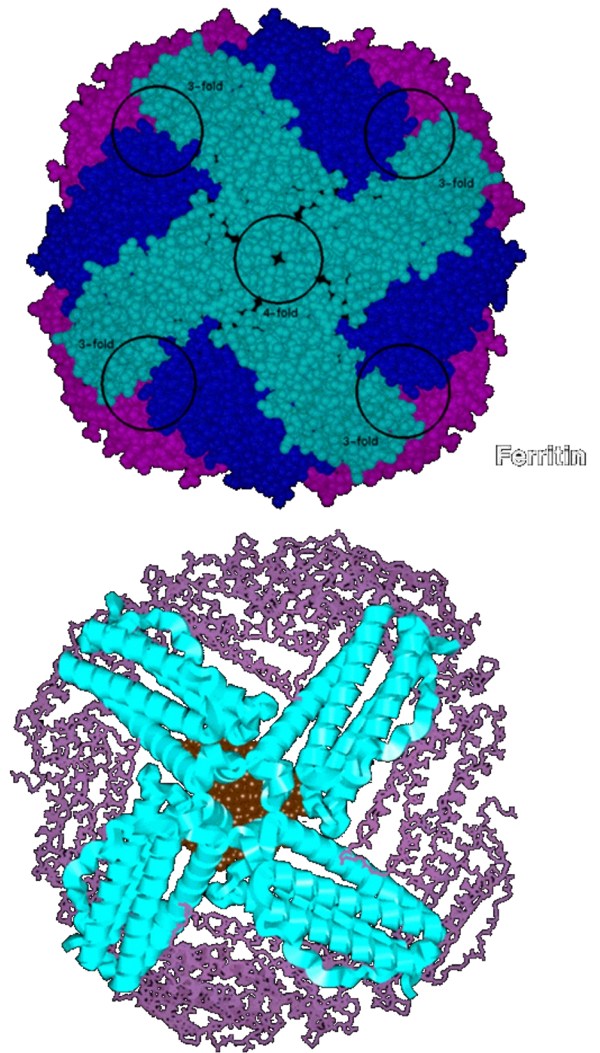
Tomato Bushy Stunt Virus 1980



I. K. Robinson Astbury 2007

Horse Spleen Ferritin

- Ferritin used in almost all living things for Iron Storage
- Made of 24 identical protein subunits arranged in 432 symmetry
- Crystallizes as FCC (I23)
- Inner shell diameter 80Å
- Outer shell diameter 130Å
- Iron core: ferrihydrite form
- With Iron : **Holo**ferritin
- Empty Shell : **Apo**ferritin



Critical Nucleation

Yau S.-T. and Vekilov P.G., *J. Am. Chem. Soc.* (2001), 123, 1080-1089

- a) Nucleation via planar 2D clustering
- b) Nucleation via compact close-packed 3D clustering
- Possible also via non-crystalline (eg icosahedral) nuclei.

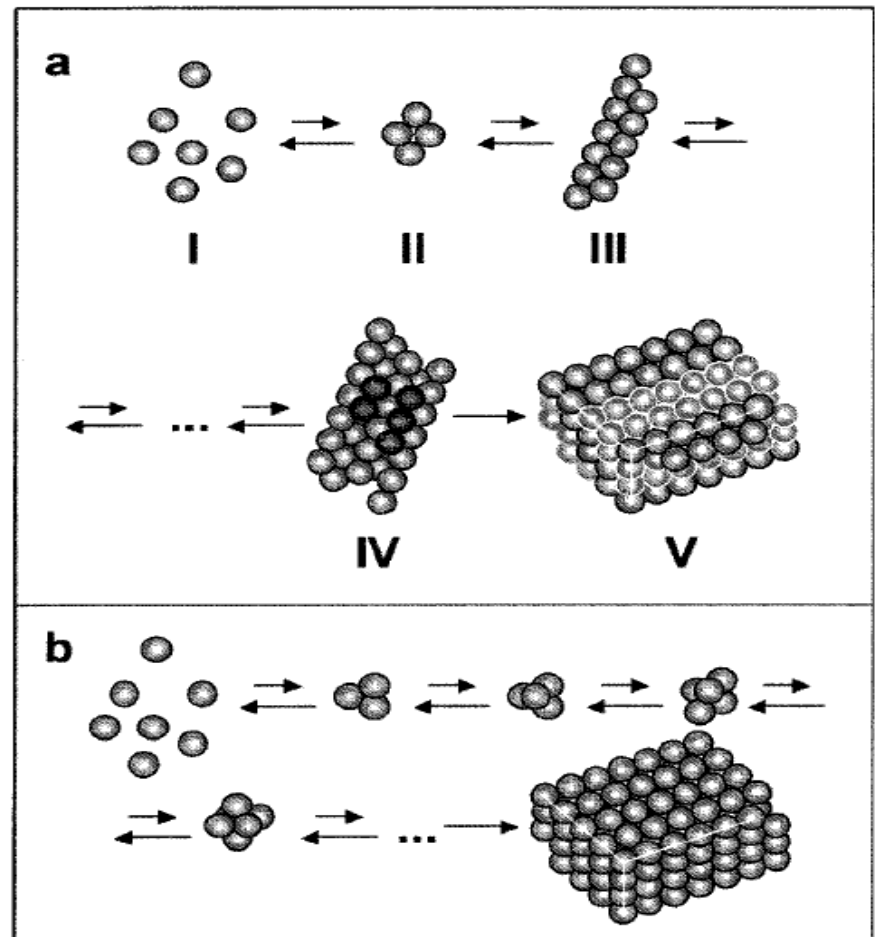
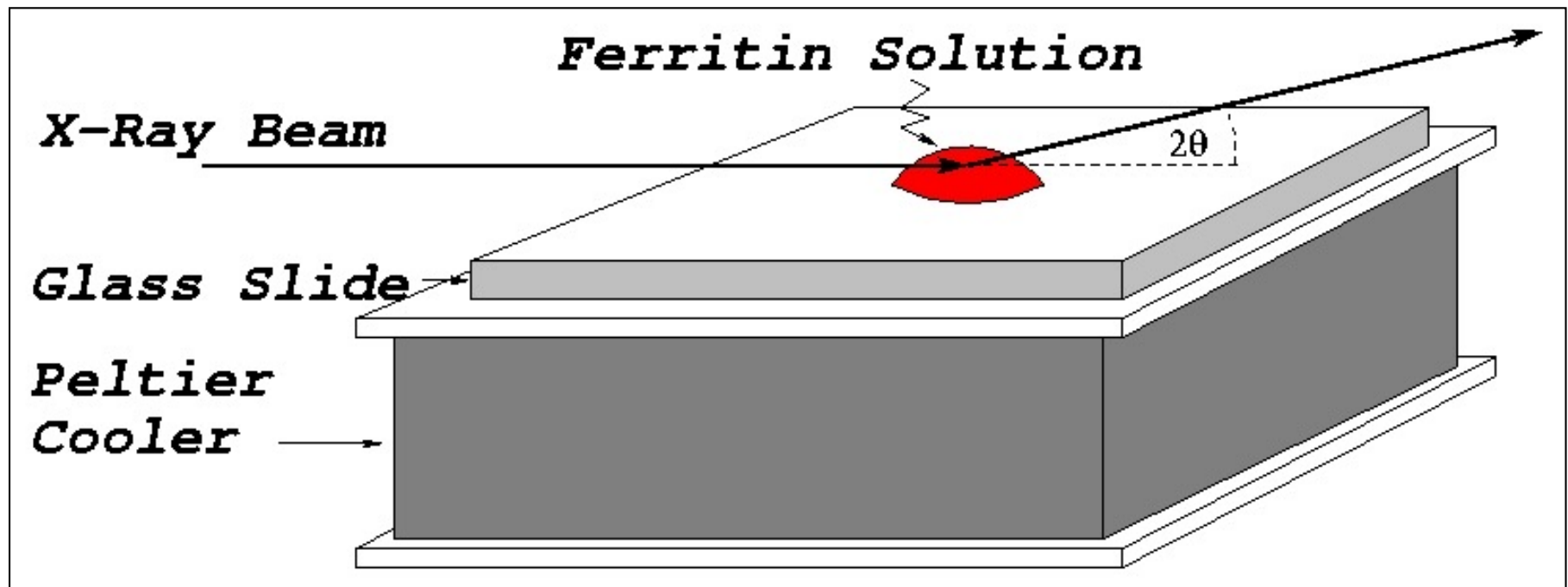
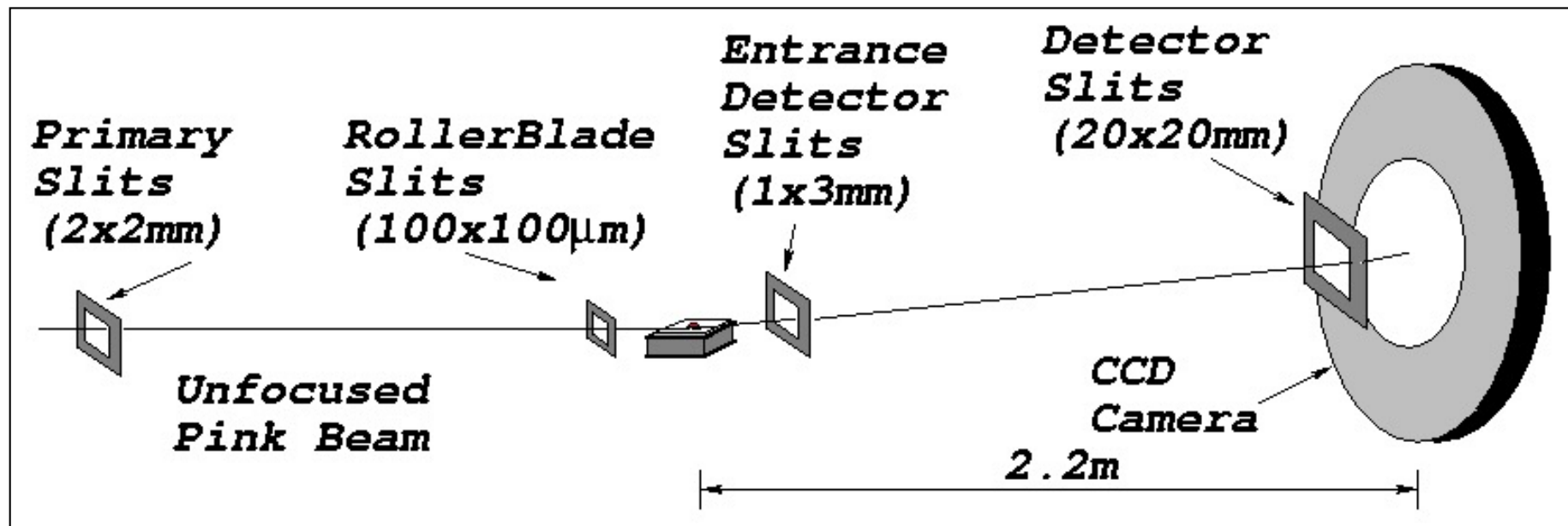


Figure 10. Schematic illustration of two nucleation pathways: (a) via a planar critical cluster (in IV molecules belonging to the second layer are shown in a lighter shade; in V the (110) layers that stack up to form this crystal are delineated by lighter and darker contours) and (b) via compact critical cluster.^{5,6}

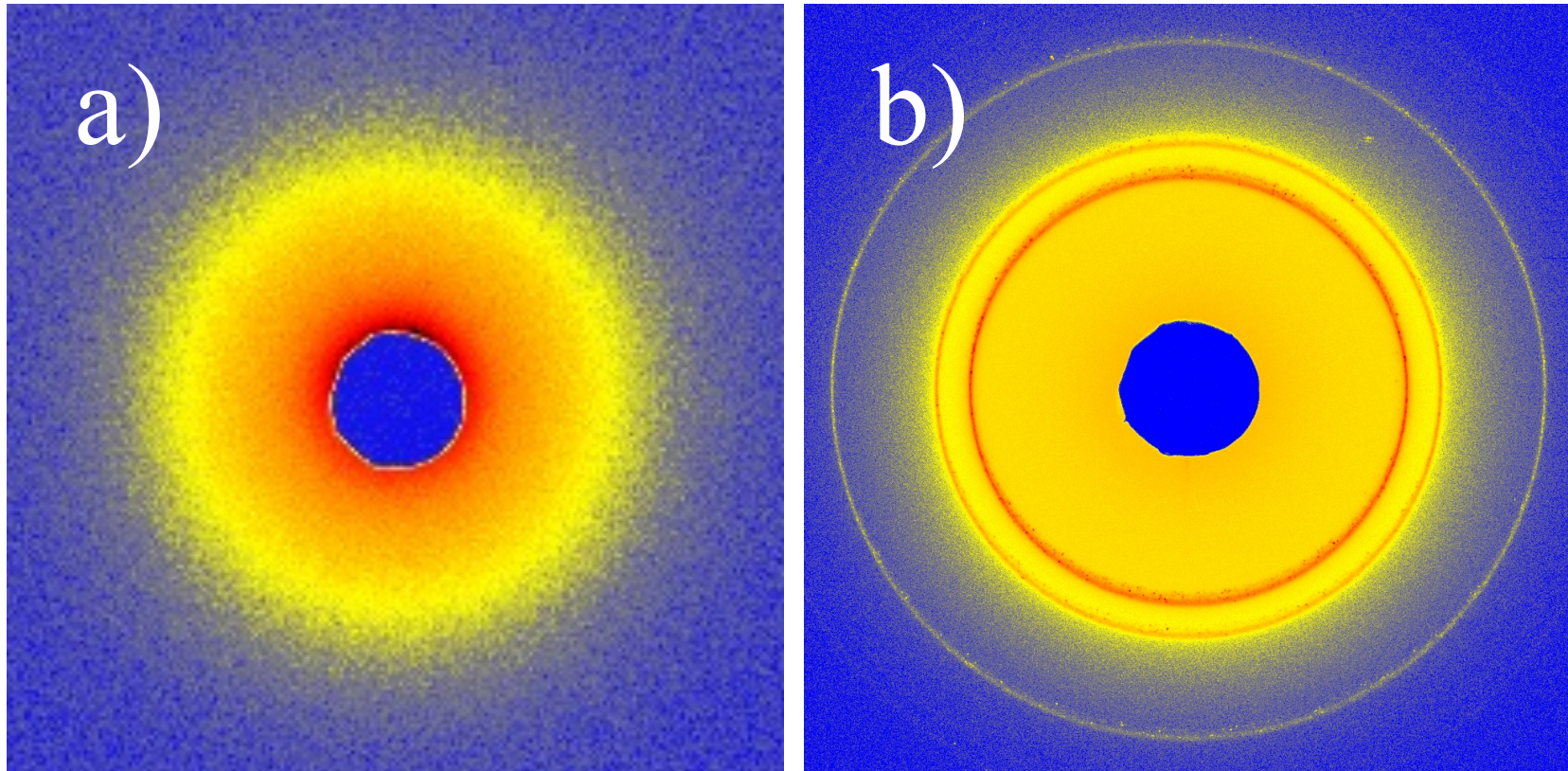
In-situ Study of Crystallization



Experiment at APS Sector 34



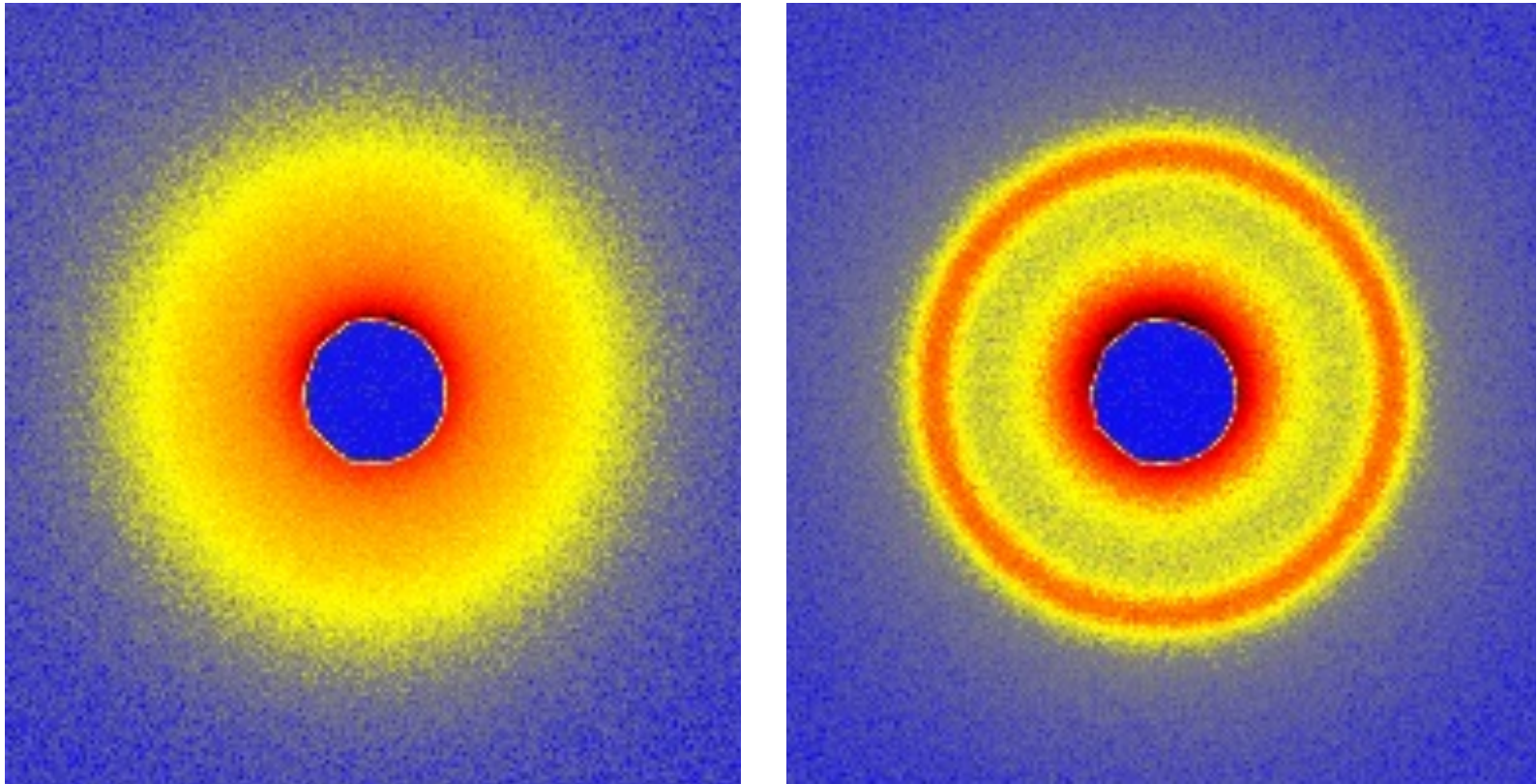
Crystallization of Holoferitin: SAXS



a) SAXS pattern of holoferitin at 10°C.

b) SAXS pattern after adding Cadmium salt

Effect of Temperature

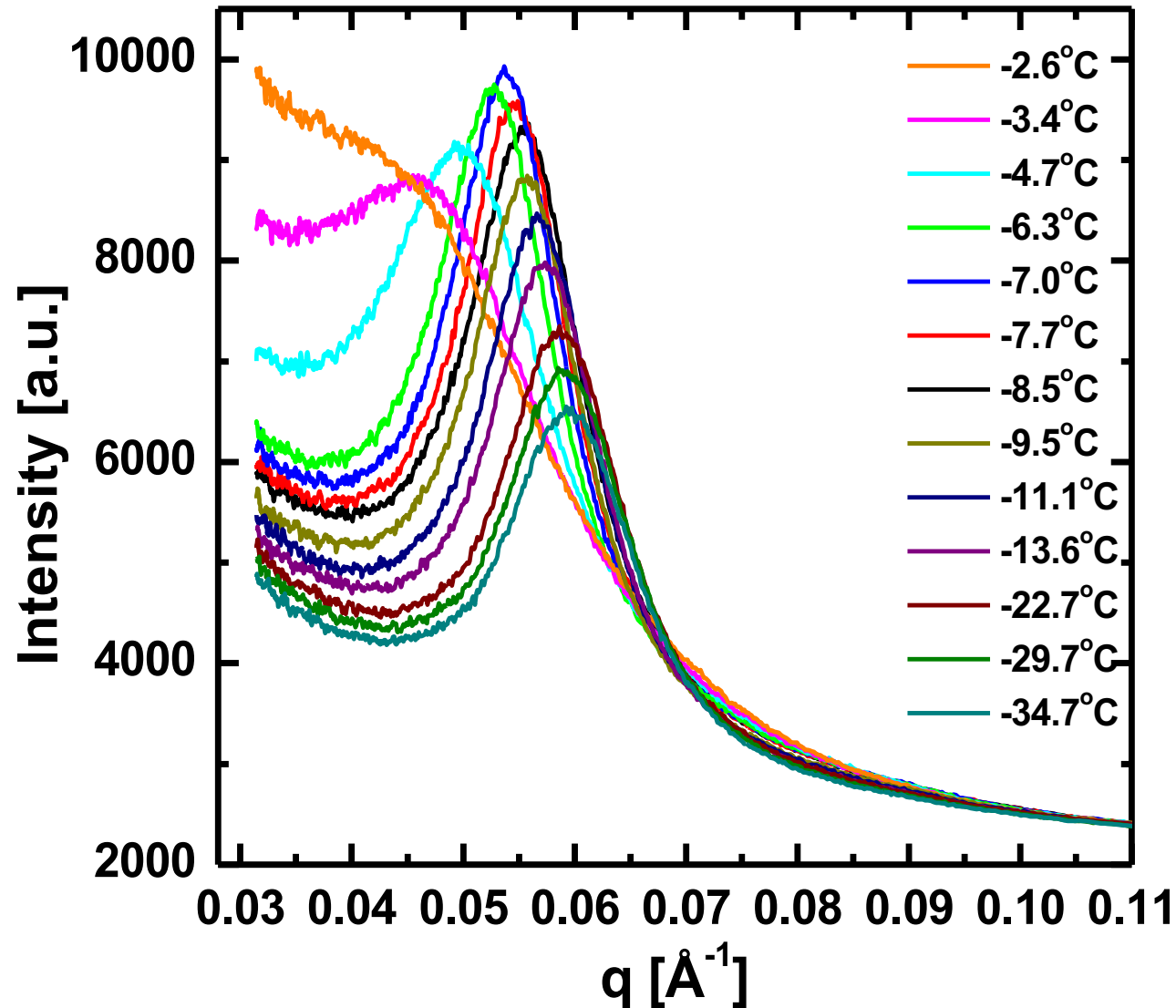


SAXS pattern at $T=+10^{\circ}\text{C}$ and -10°C .

Holoferritin in dilute (10mg/ml) NaCl only.

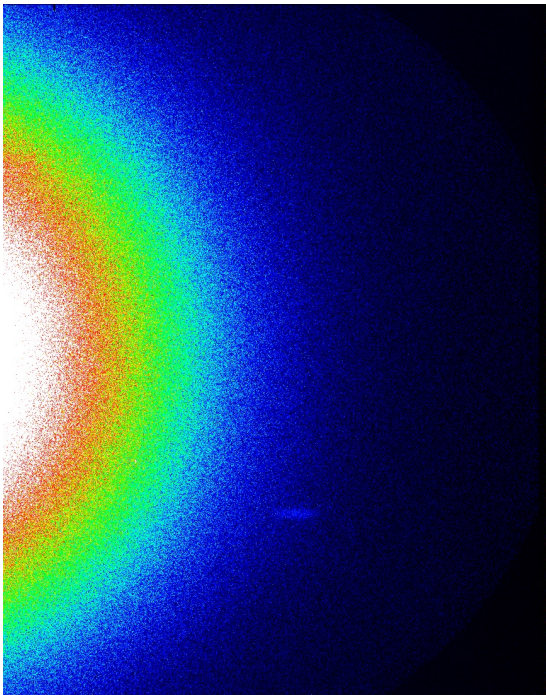
I. K. Robinson Astbury 2007

Integrated SAXS Data

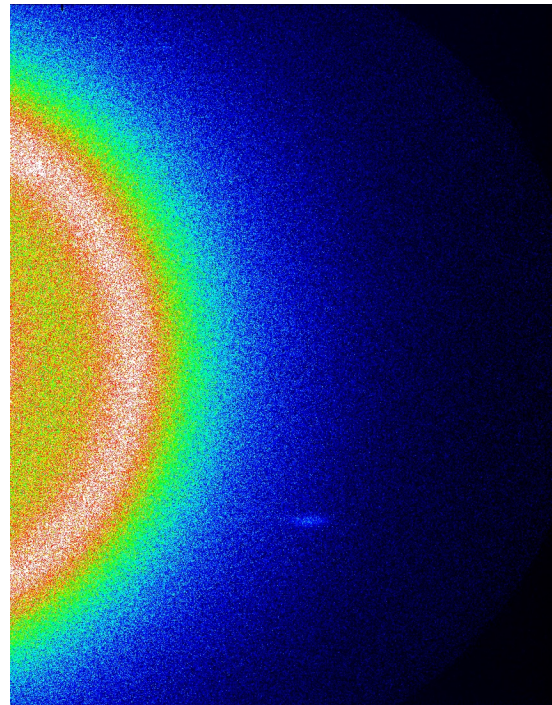


- The SAXS pattern shows a broad peak which shifts in position and changes in width as a function of temperature

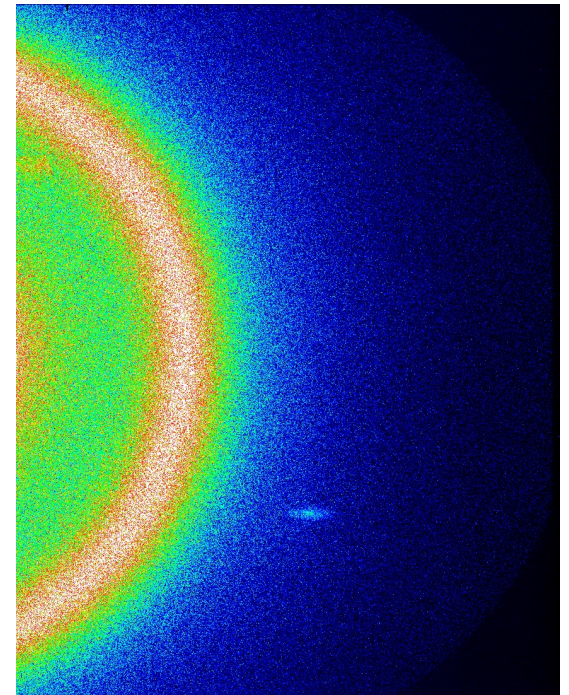
Ferritin Solution upon Freezing



T=13.3

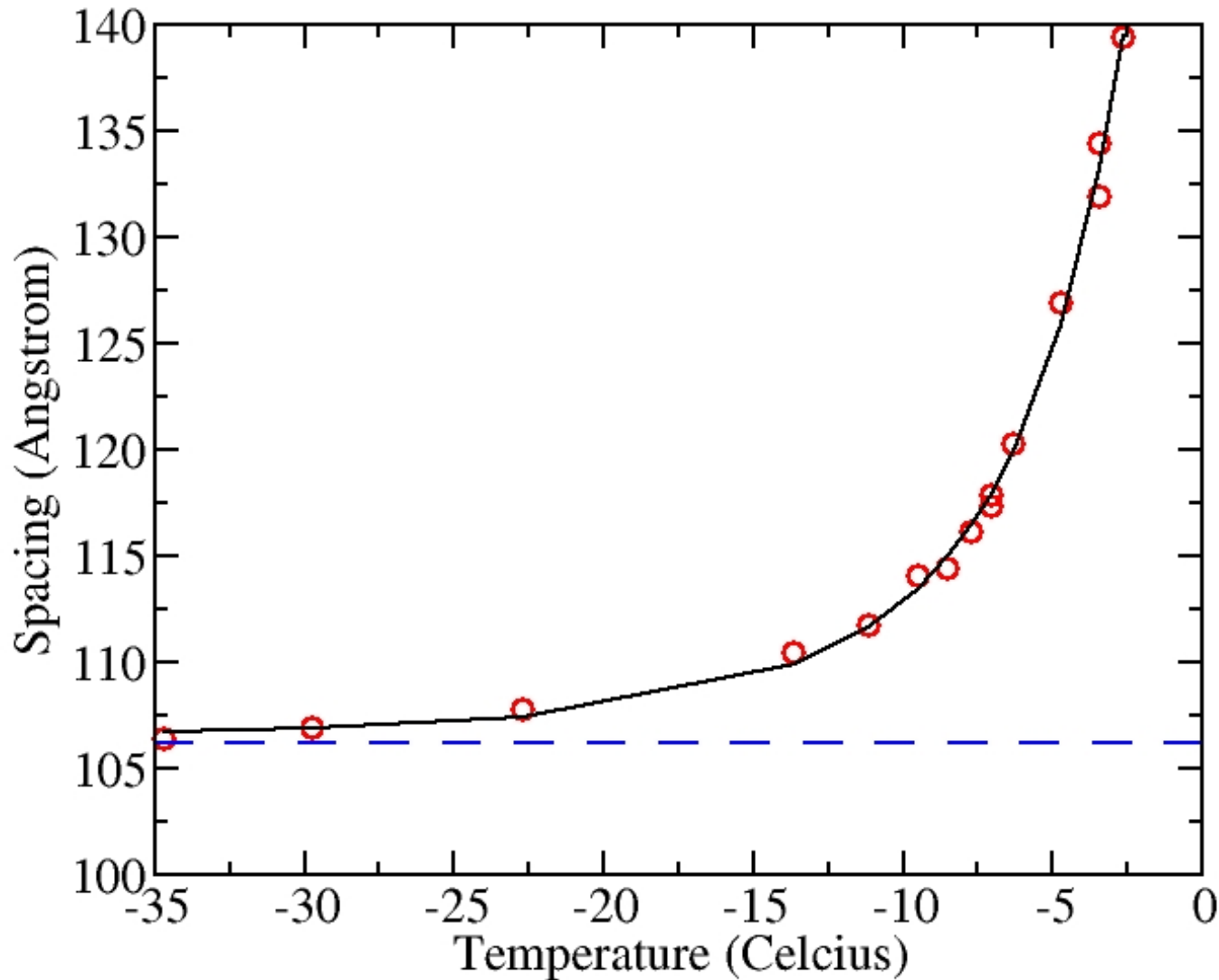


T=-4.7

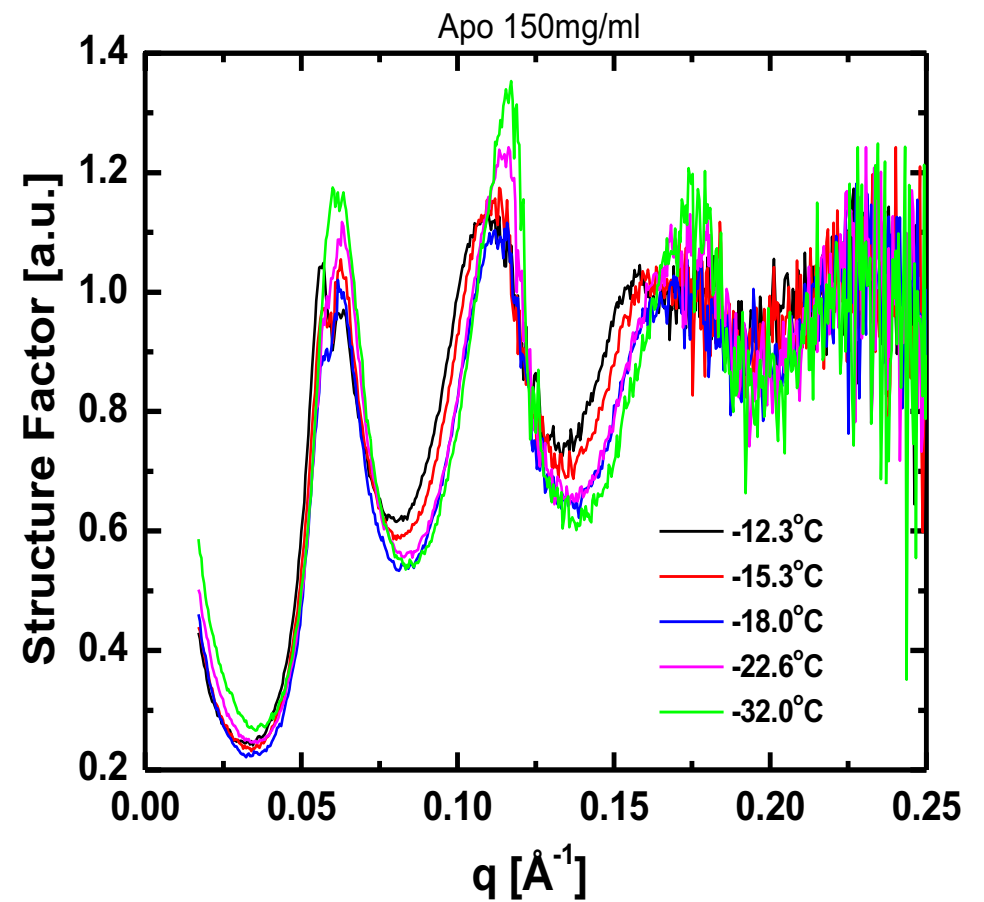
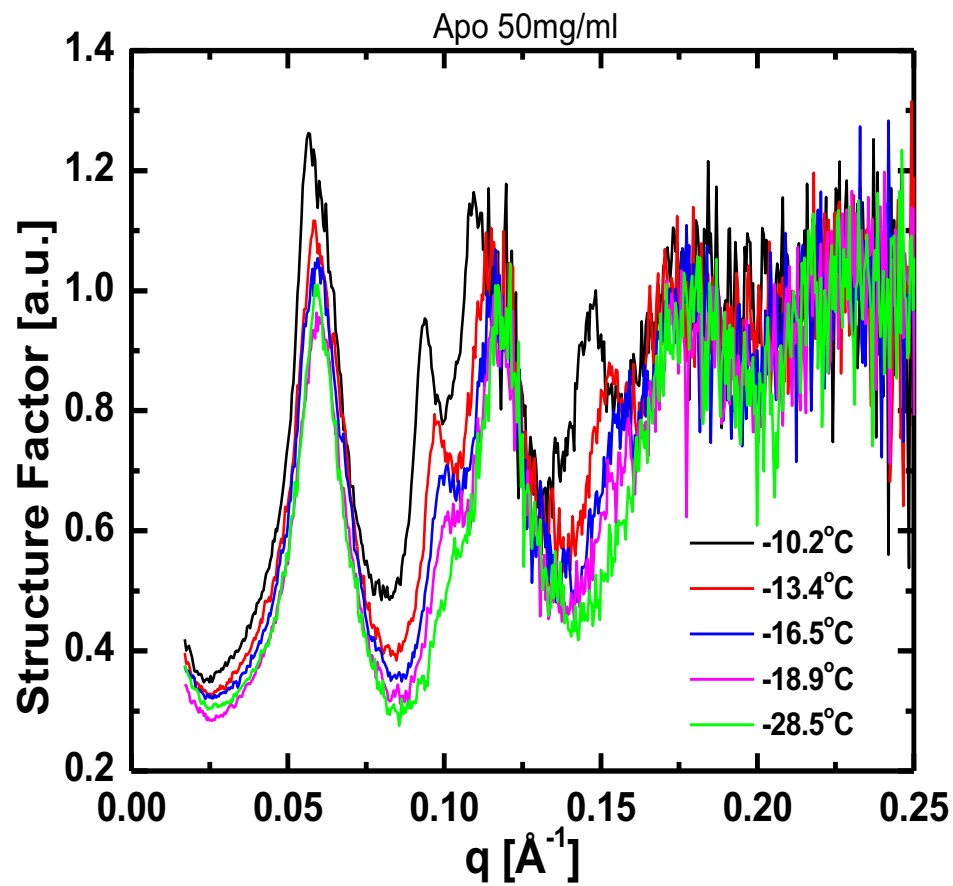


T=-34.7

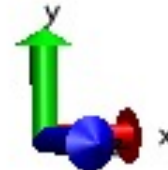
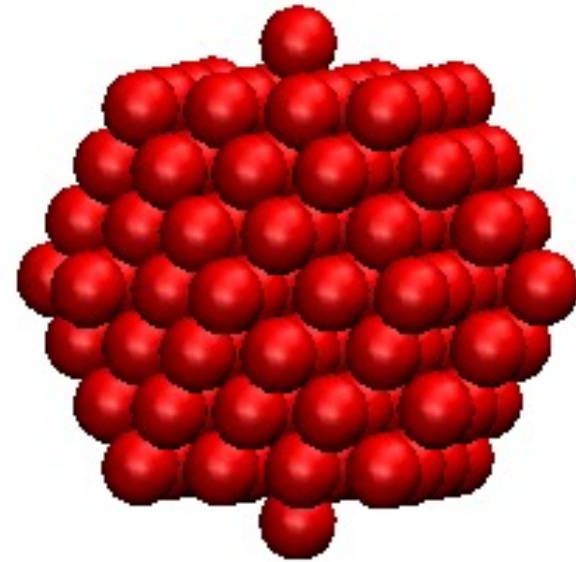
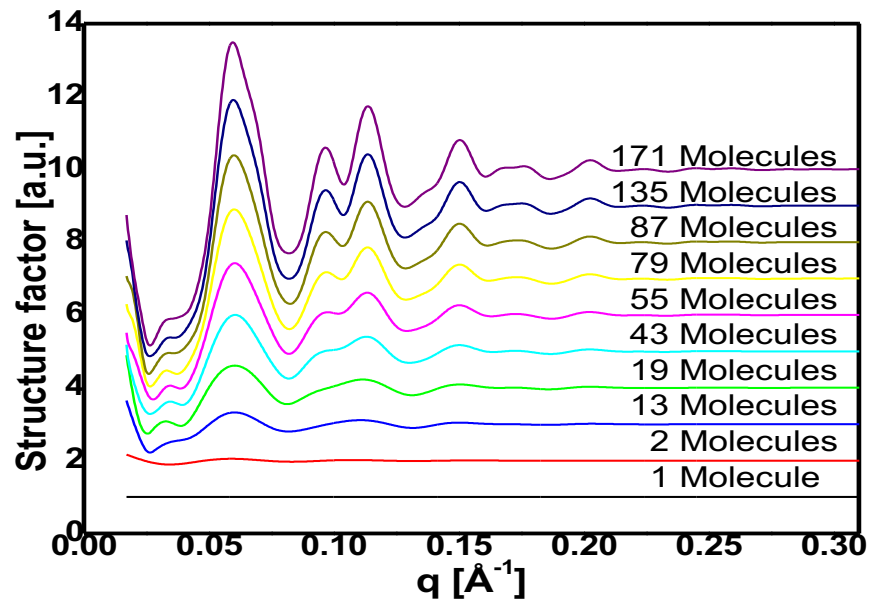
Temperature Variation of Peak Position



Structure Factors of Apoferritin

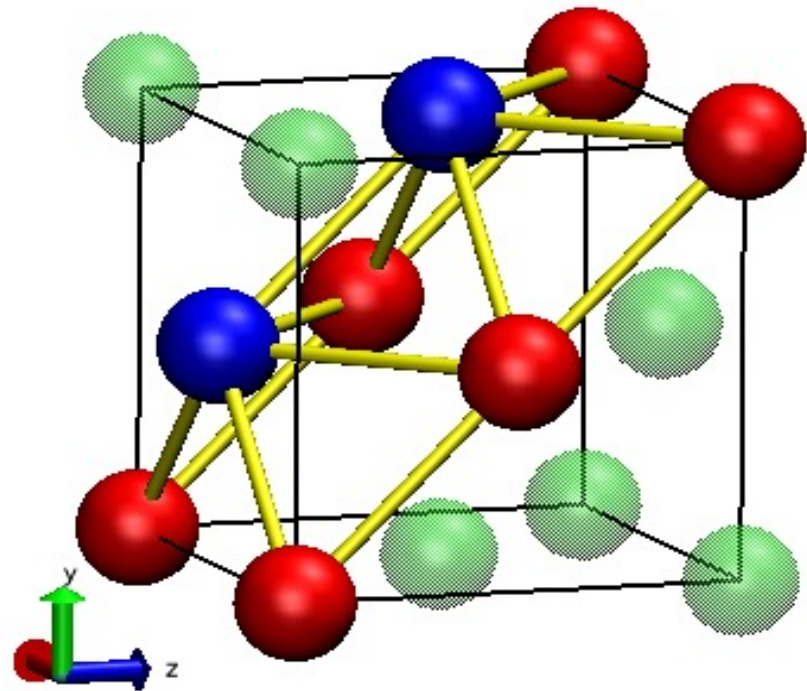
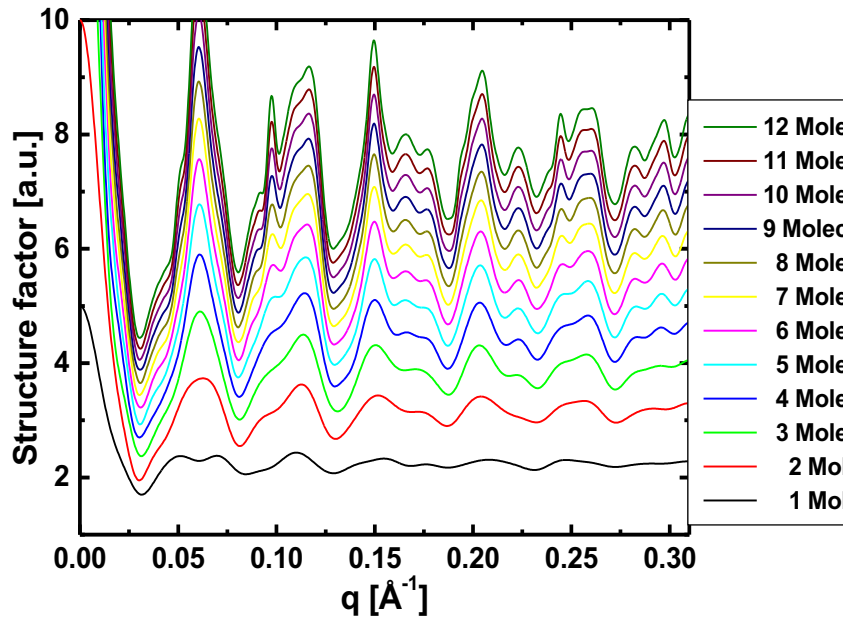


FCC Spherical Clusters



Planar Clusters of 110 Rods

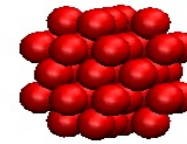
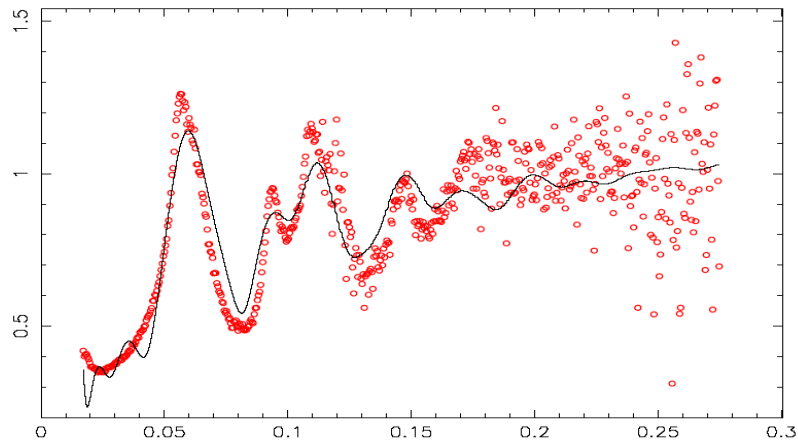
Structure factor simulation as 5 $\langle 110 \rangle$ rods with
1 to 12 molecules per rod



Best Fits

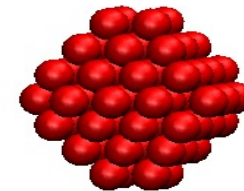
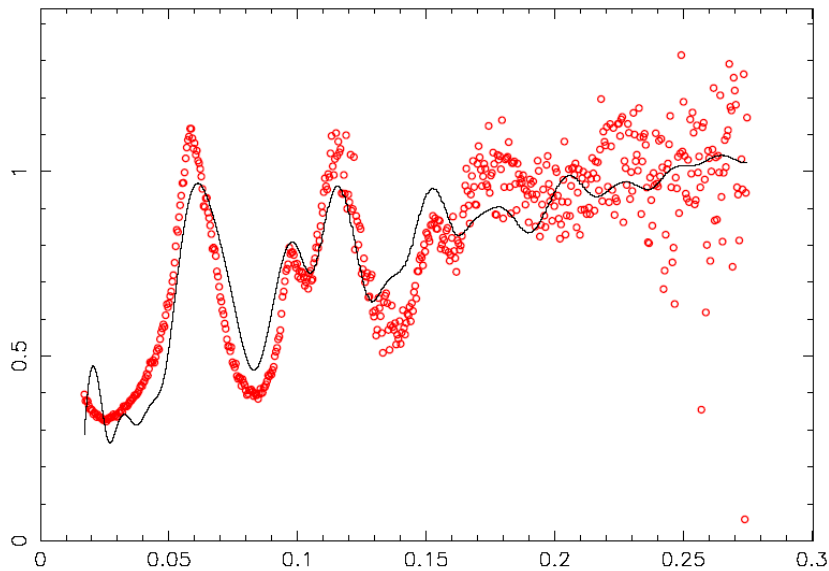
$T = -10^{\circ}\text{C}$

Cluster Size = 36



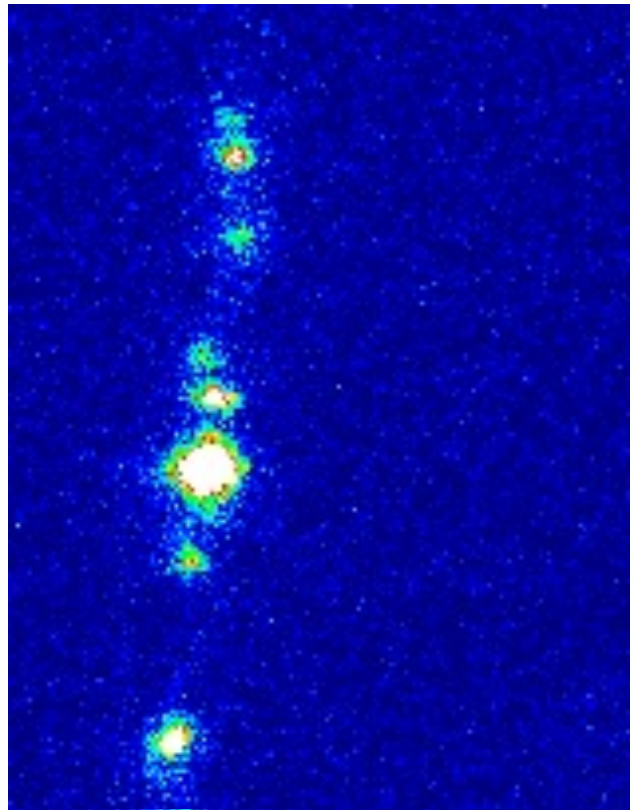
$T = -13^{\circ}\text{C}$

Cluster Size = 63

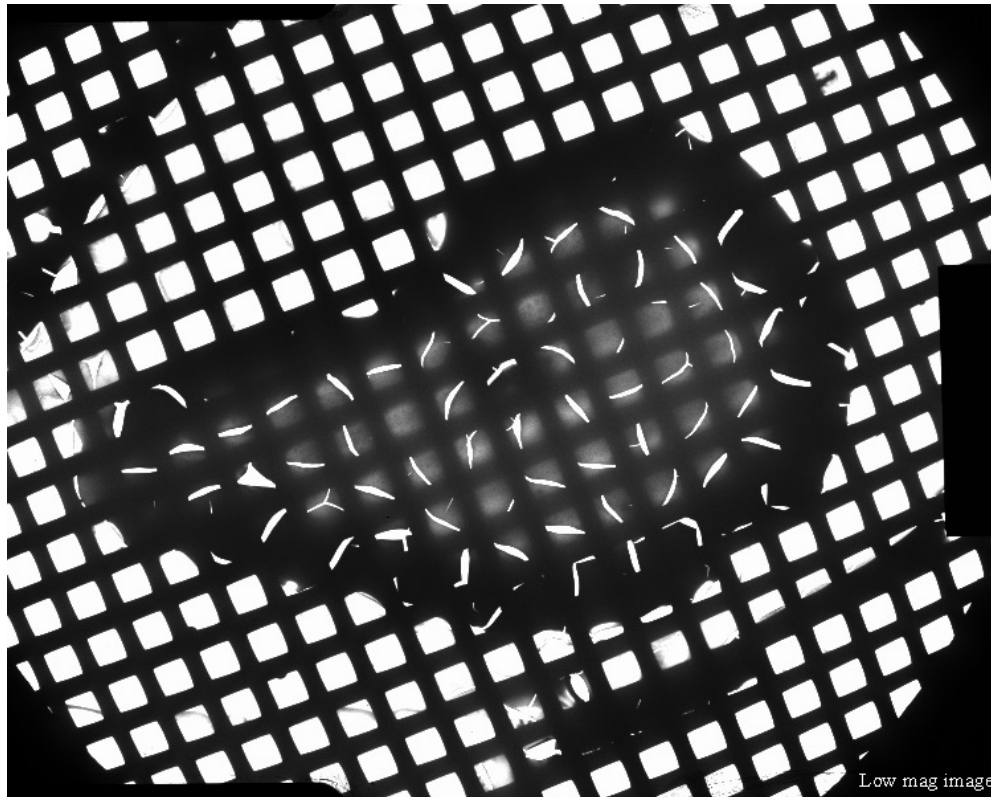


Ferritin (111) Powder Ring

- 50 frames
- 30sec exposure
- 0.3sec playback
- 150x200 pixels of 22.5 μm

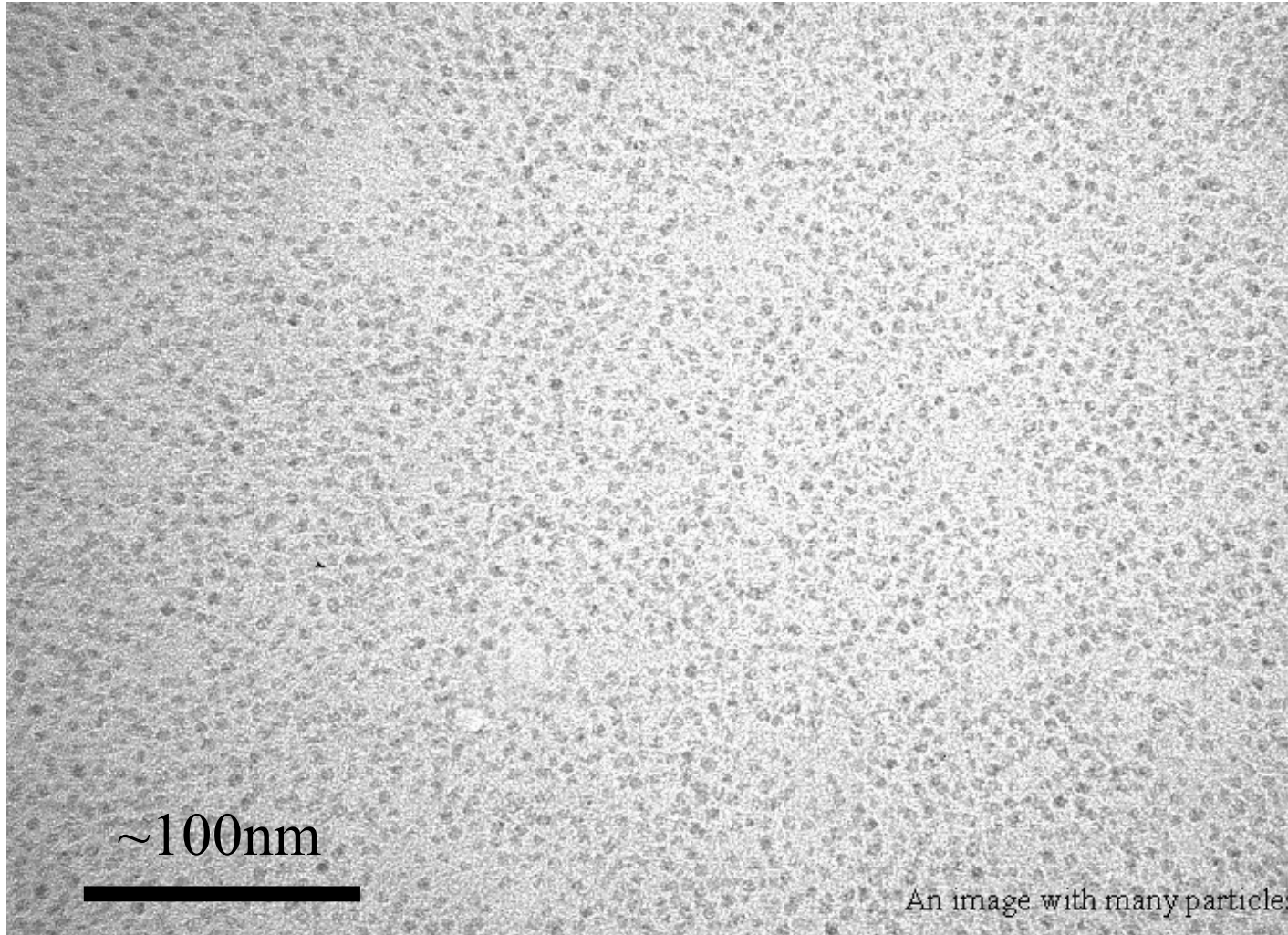


Overview of EM grid



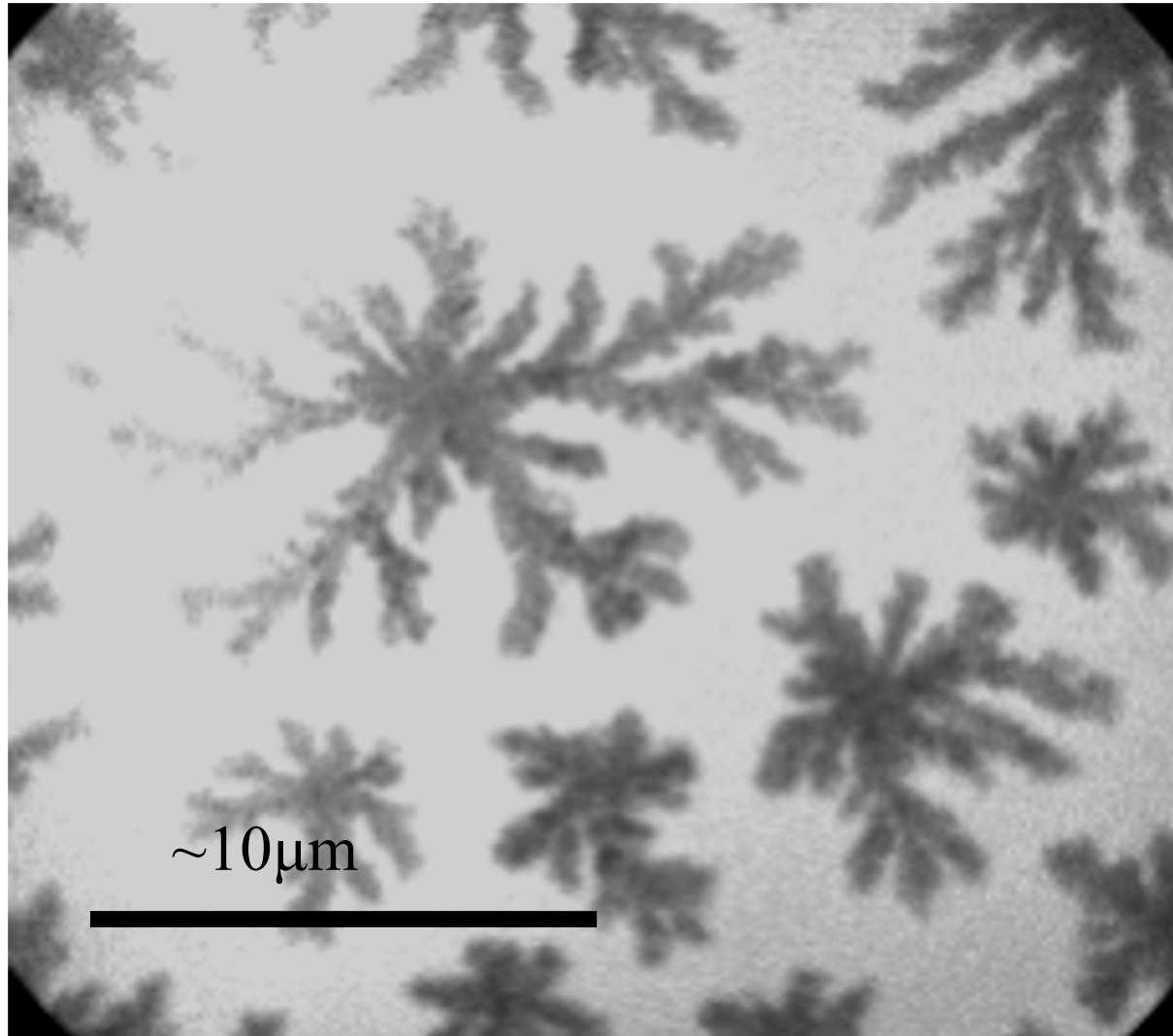
- 3mm diam grid
- a-carbon + “Formvar”
- 10mg/ml Ferritin
- 10mg/ml NaCl
- dipped grid
- dried in air
- no cryo-cooling
 - ▷ three regions of different density

Border Region (low density)



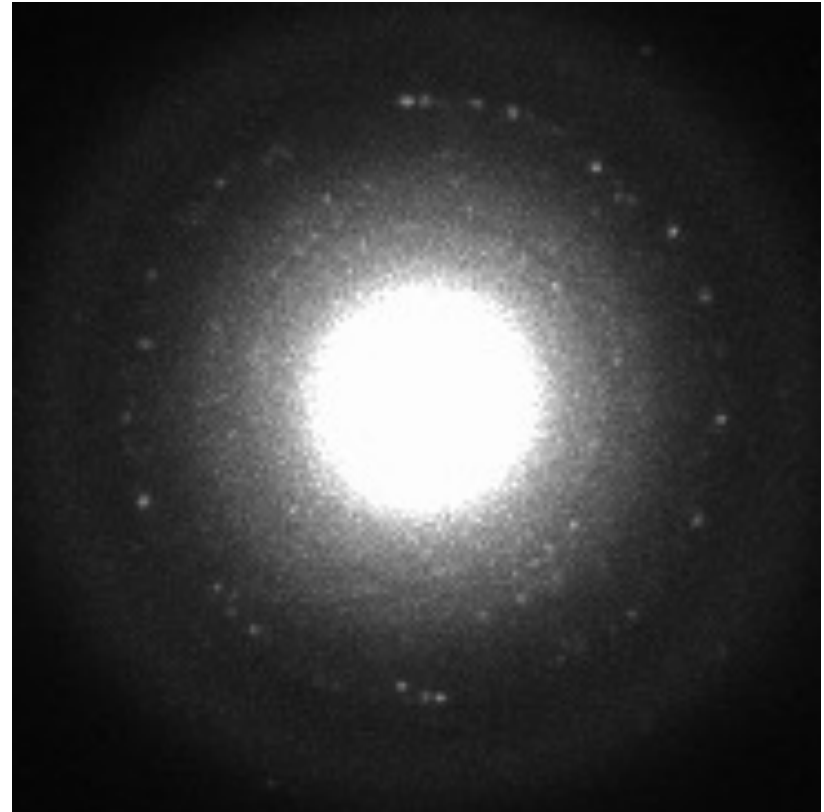
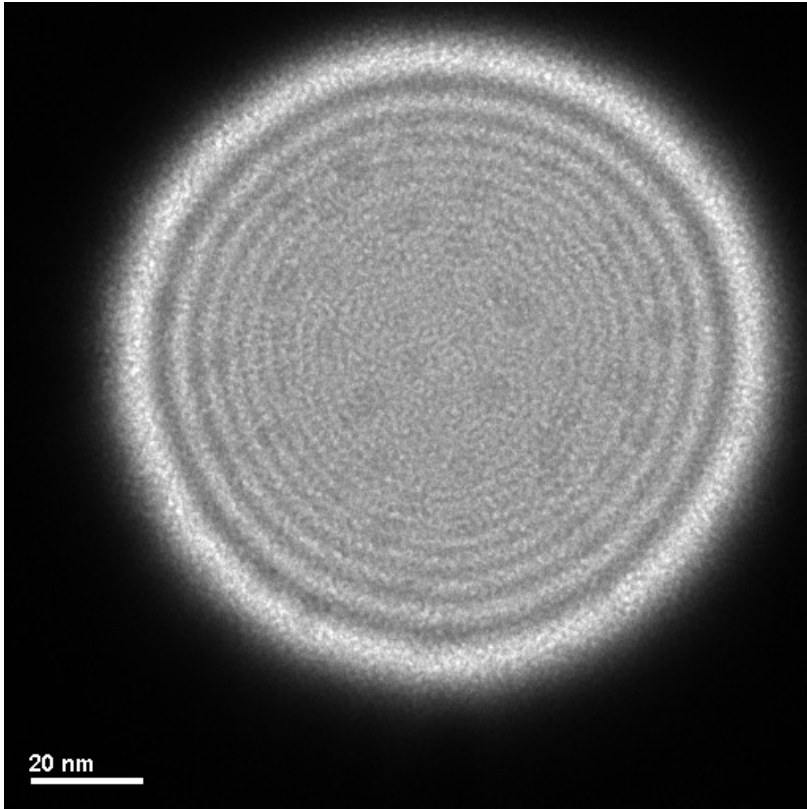
I. K. Robinson Astbury 2007

High Density Ring

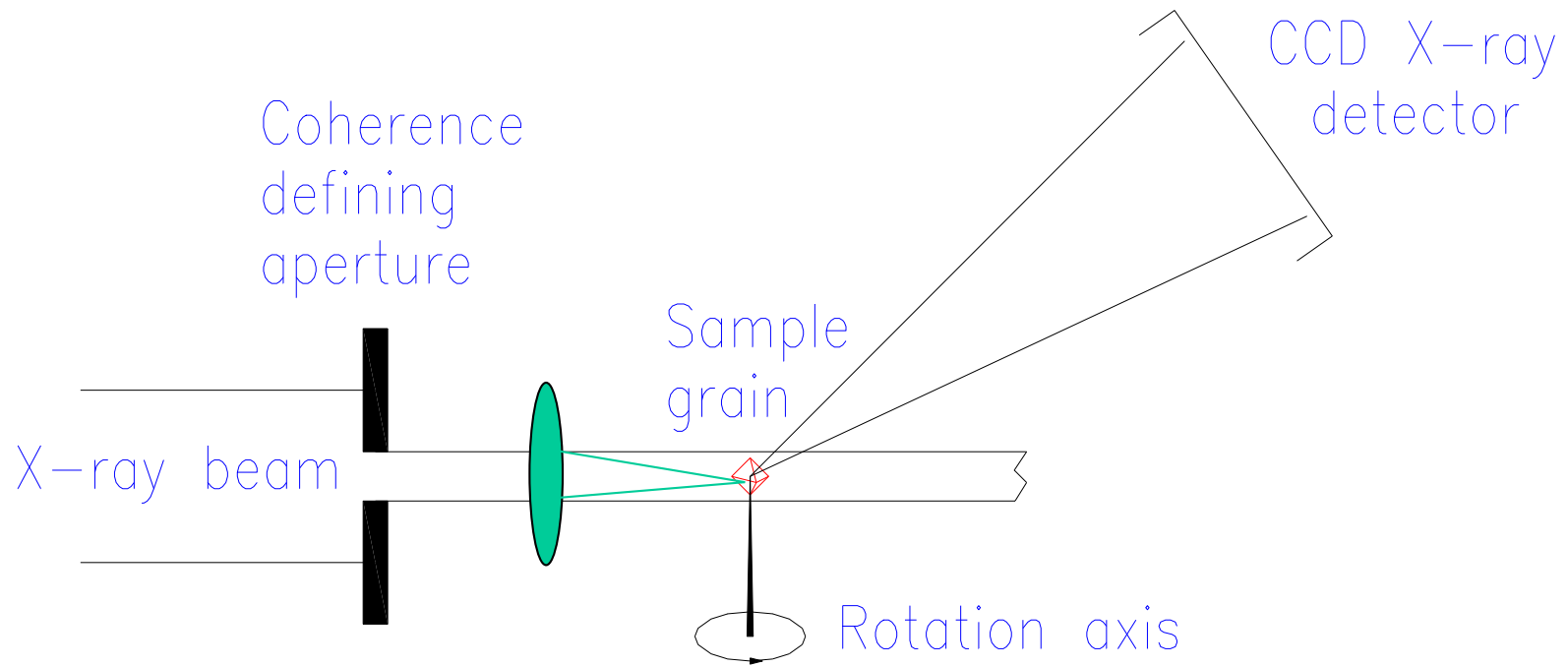


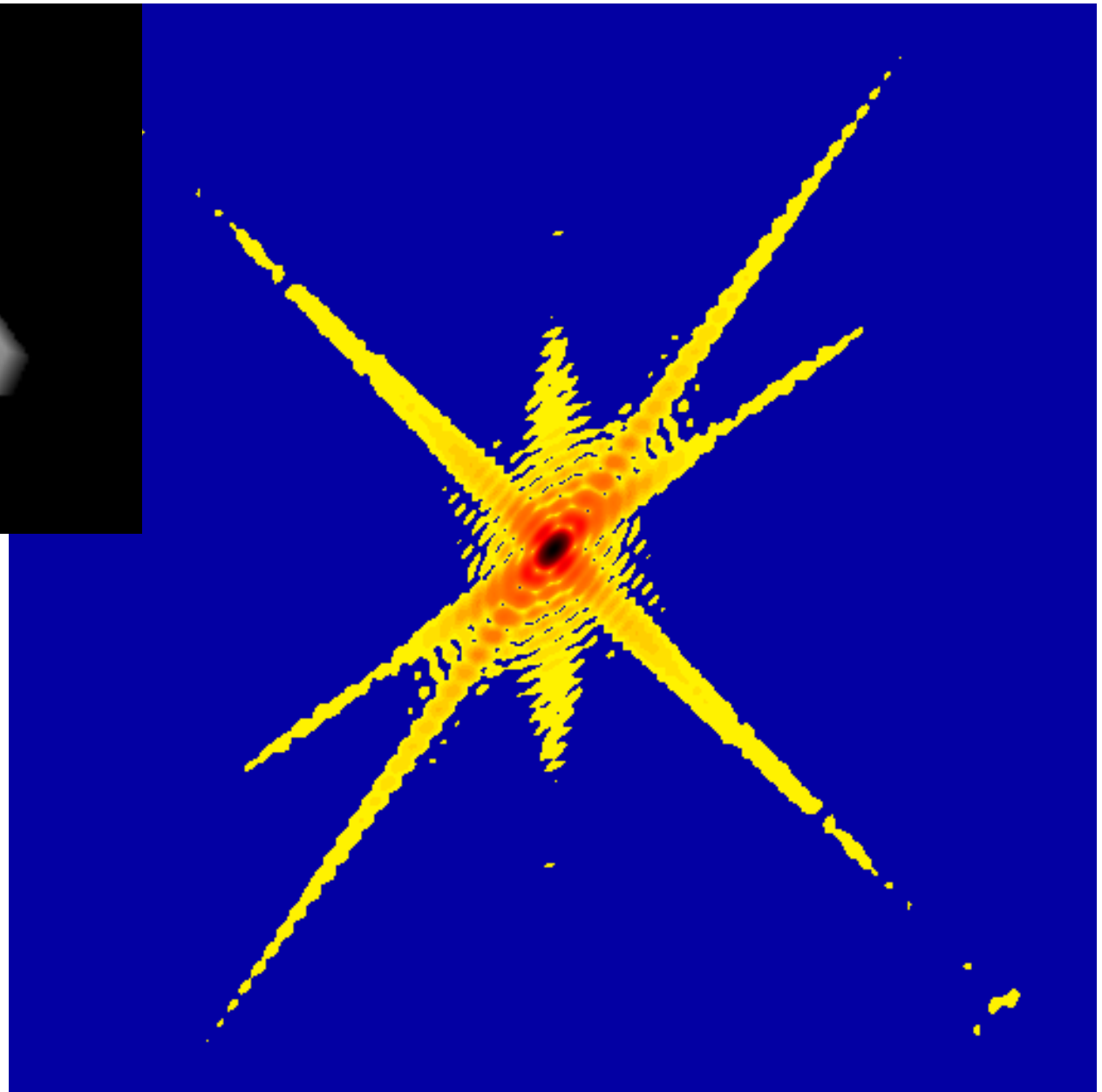
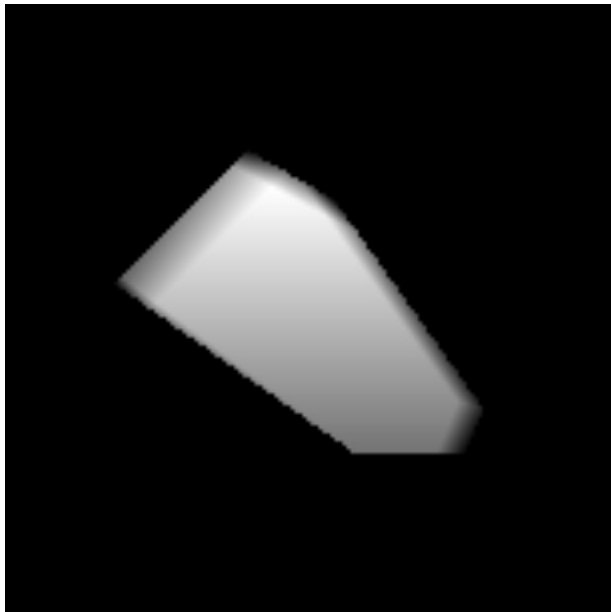
I. K. Robinson Astbury 2007

Diffraction from Border Region



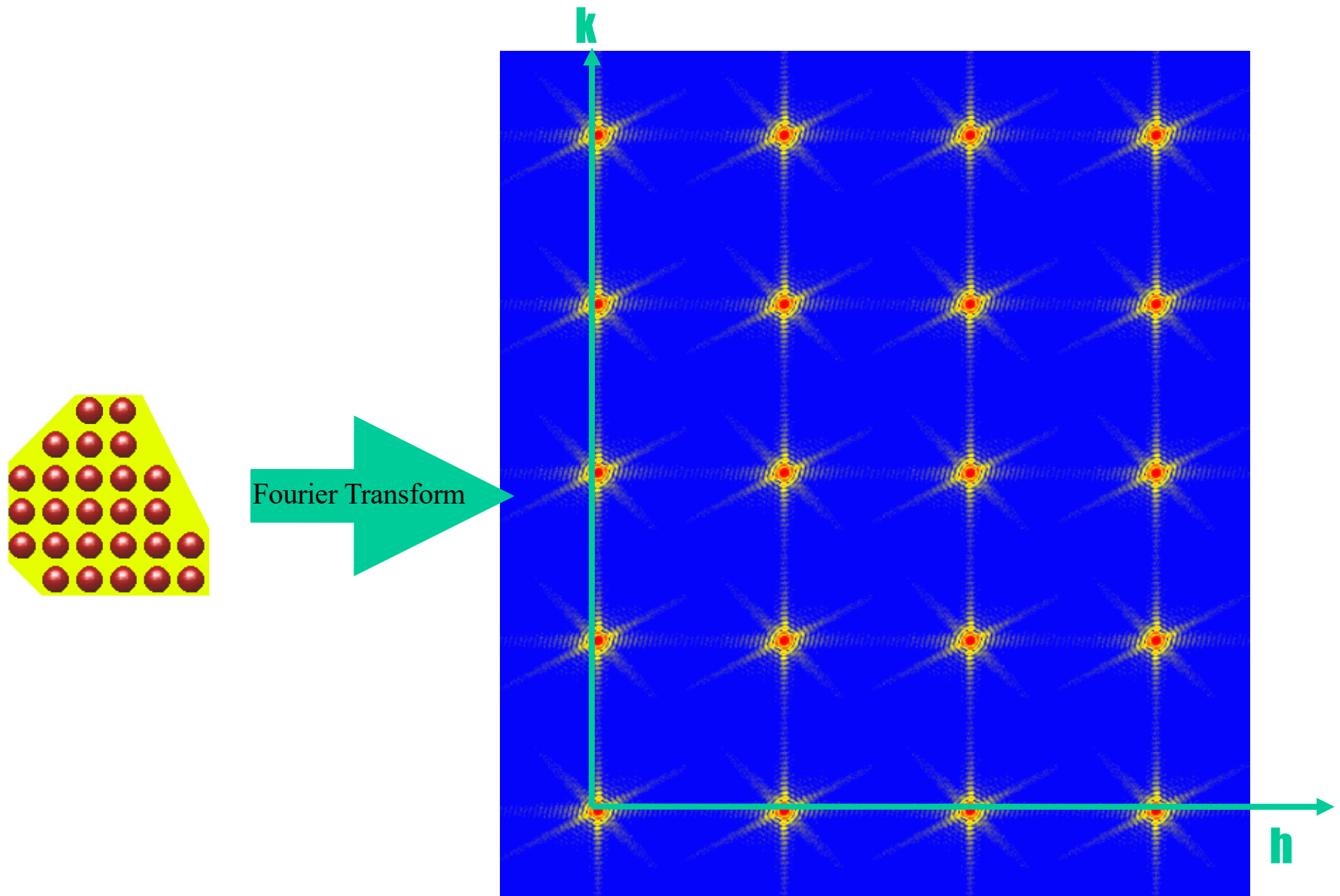
Lensless X-ray Microscope



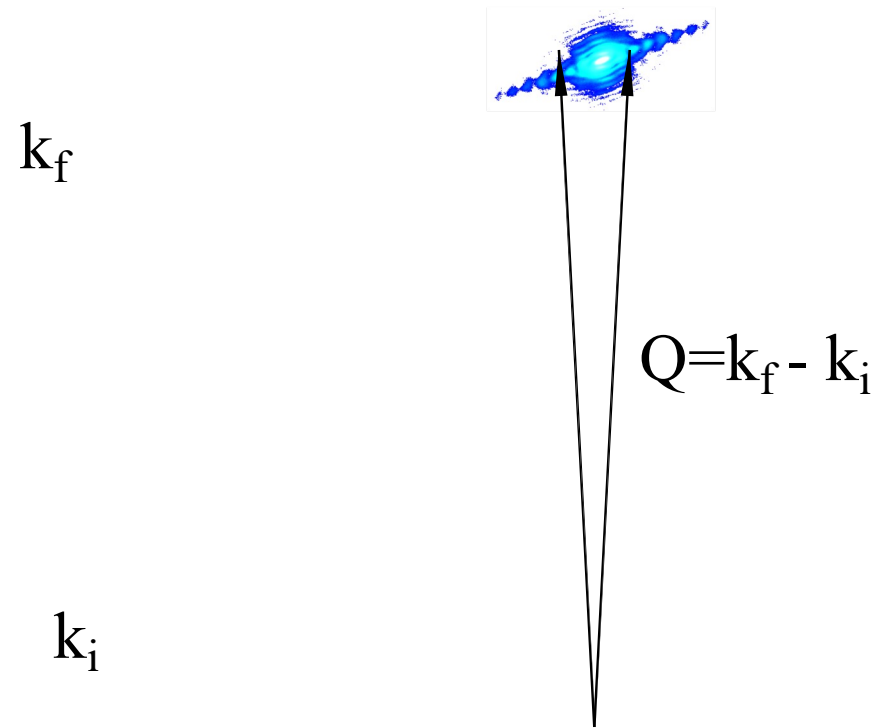


I. K. Robinson Astbury 2007

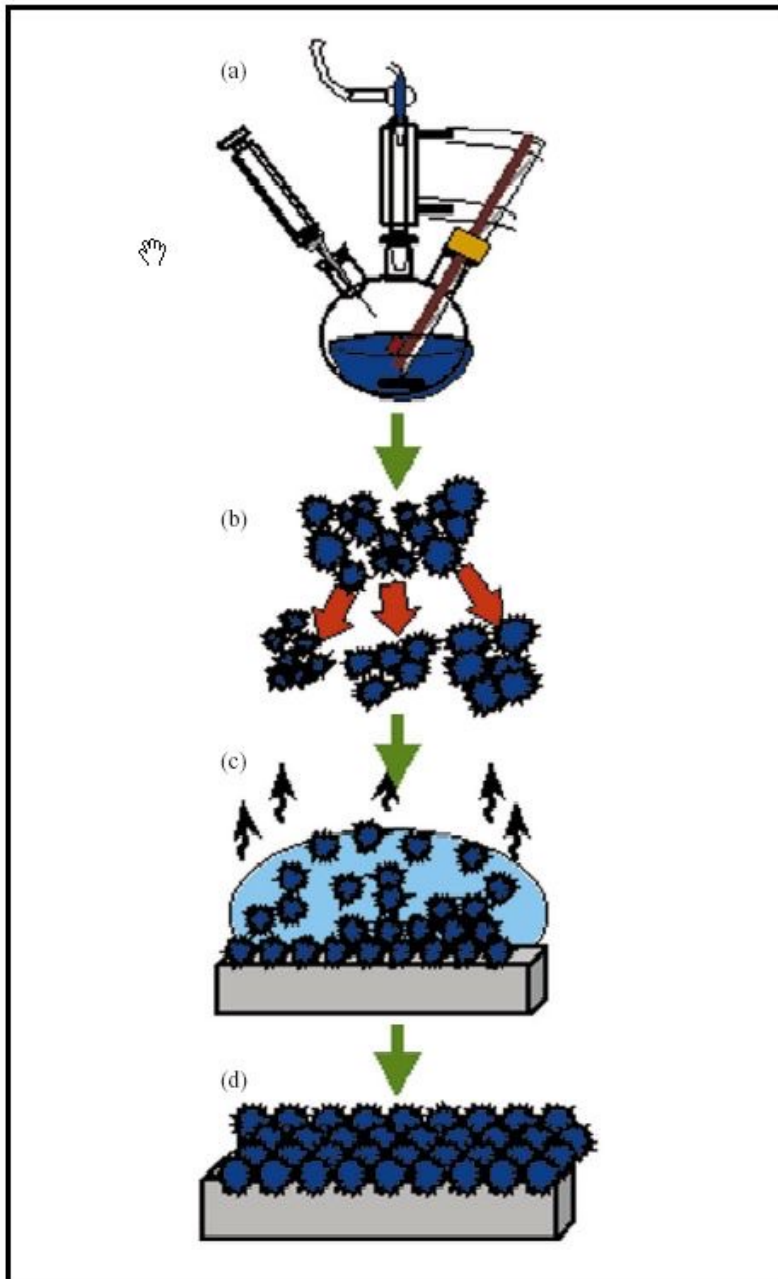
Coherent Diffraction from Crystals



3D Diffraction Method

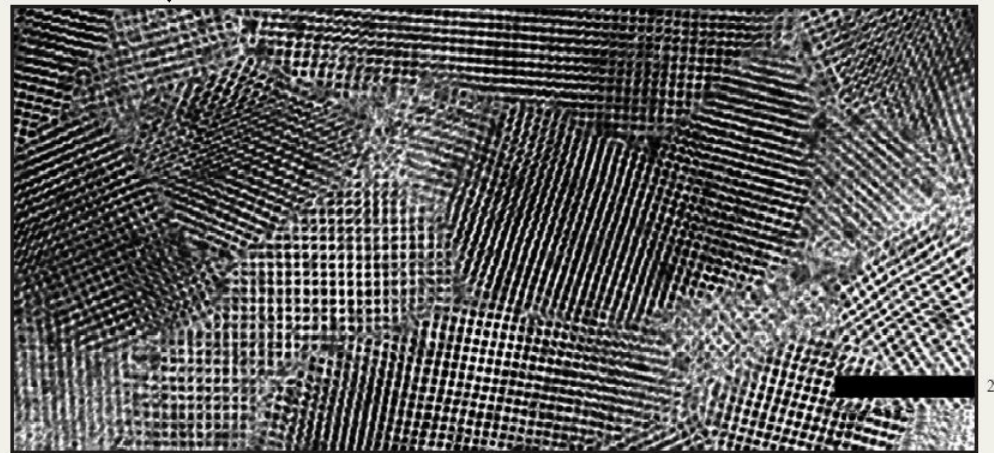


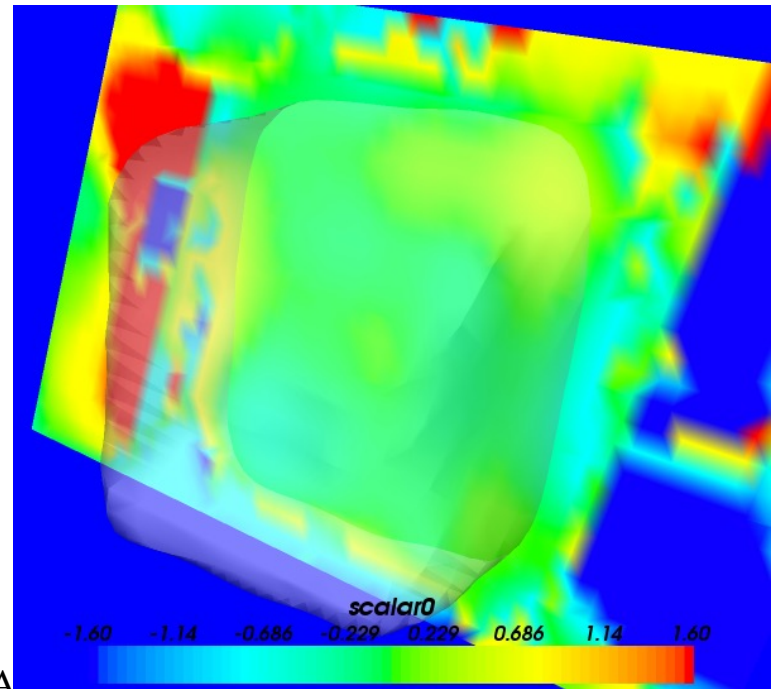
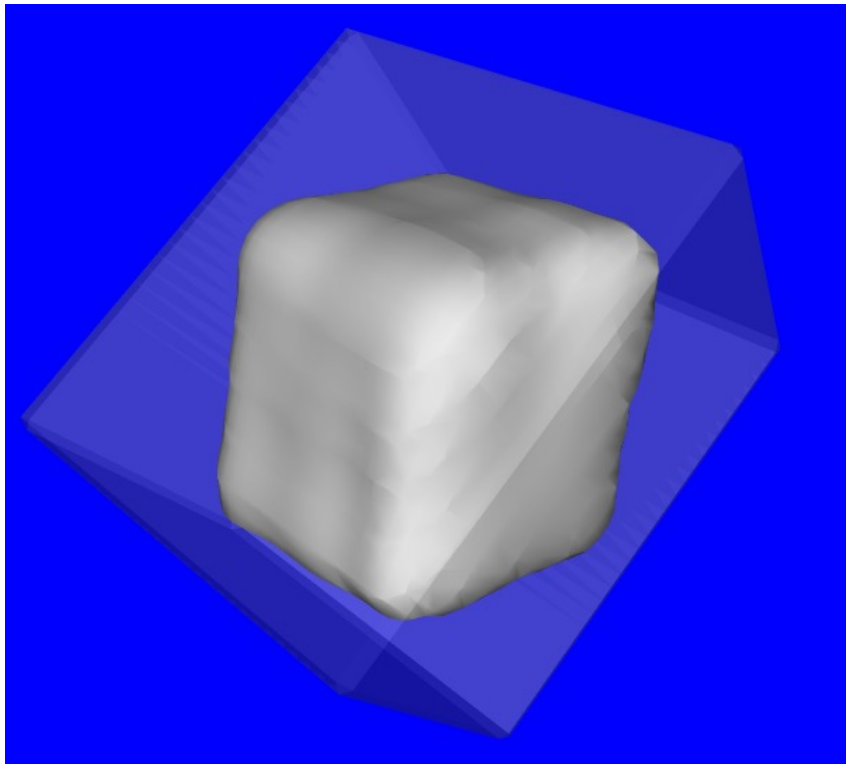
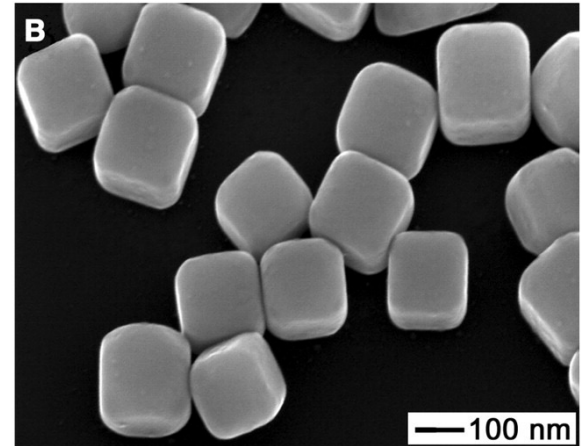
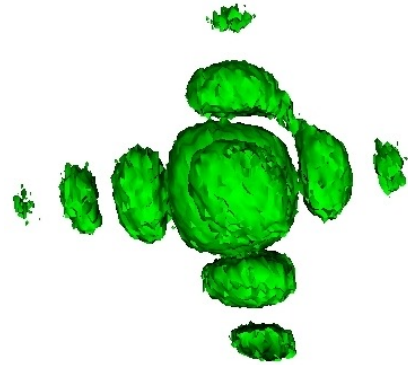
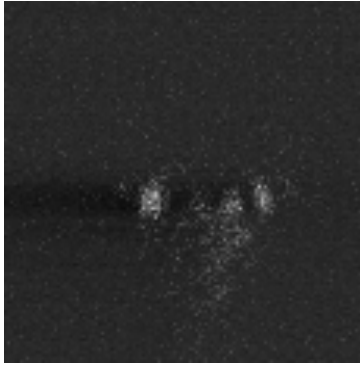
Chemical Synthesis of Nanocrystals



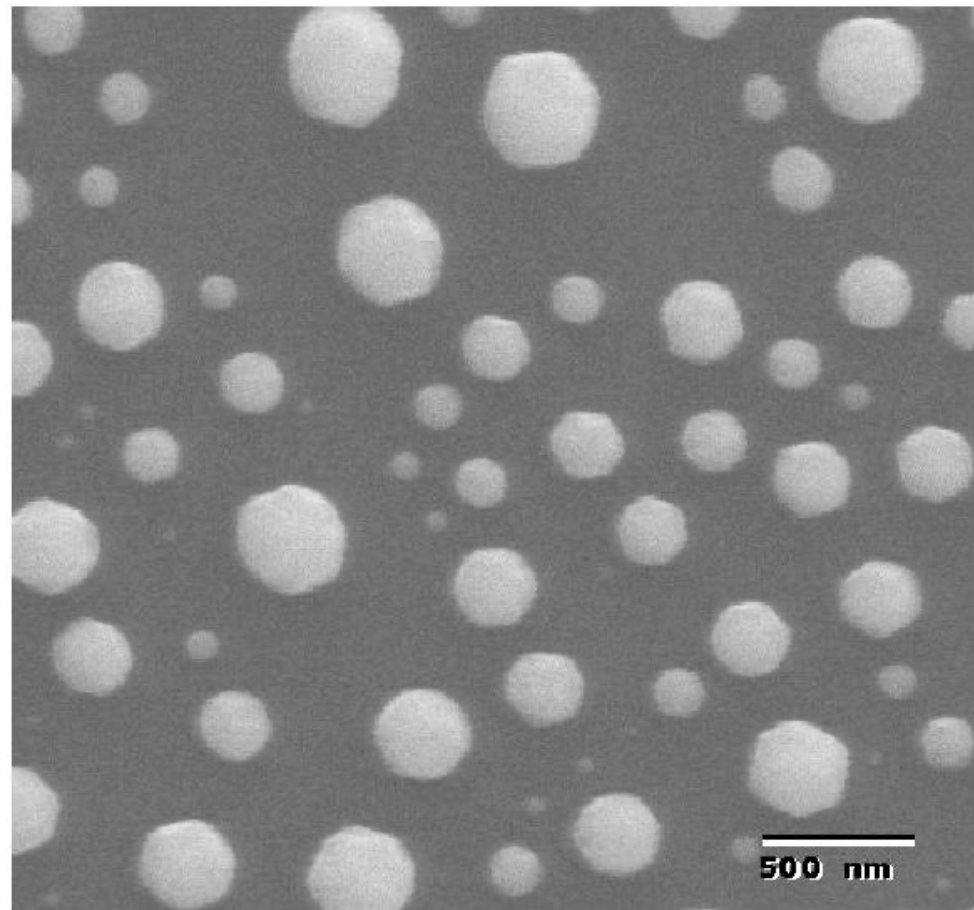
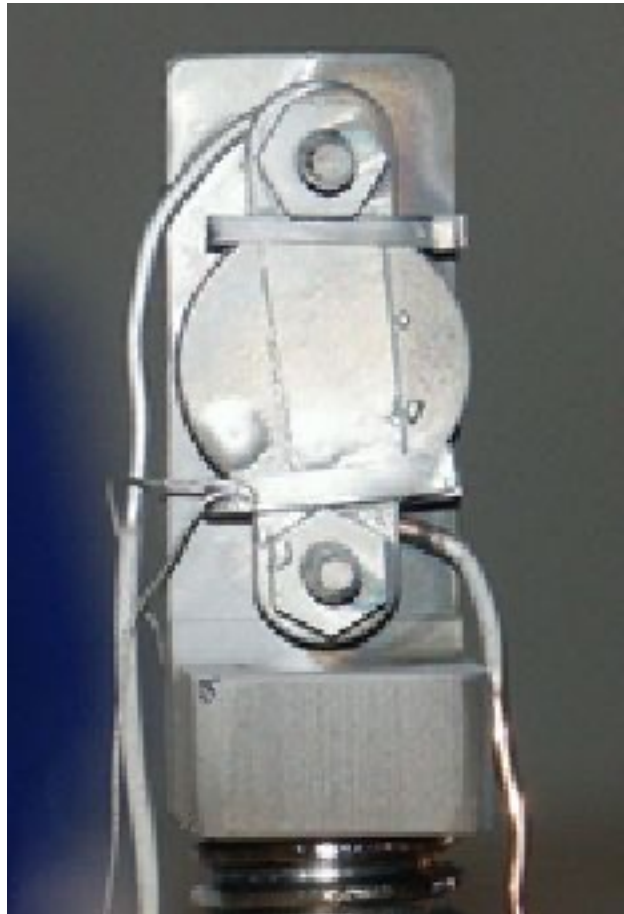
- Reactants introduced rapidly
- High temperature solvent
- Surfactant/organic capping agent
- Square superlattice (200nm scale)

C. B. Murray, IBM J. Res. & Dev. **45**
47 (2001)





In situ growth of Pb crystals



Good statistics, 3D diffraction data

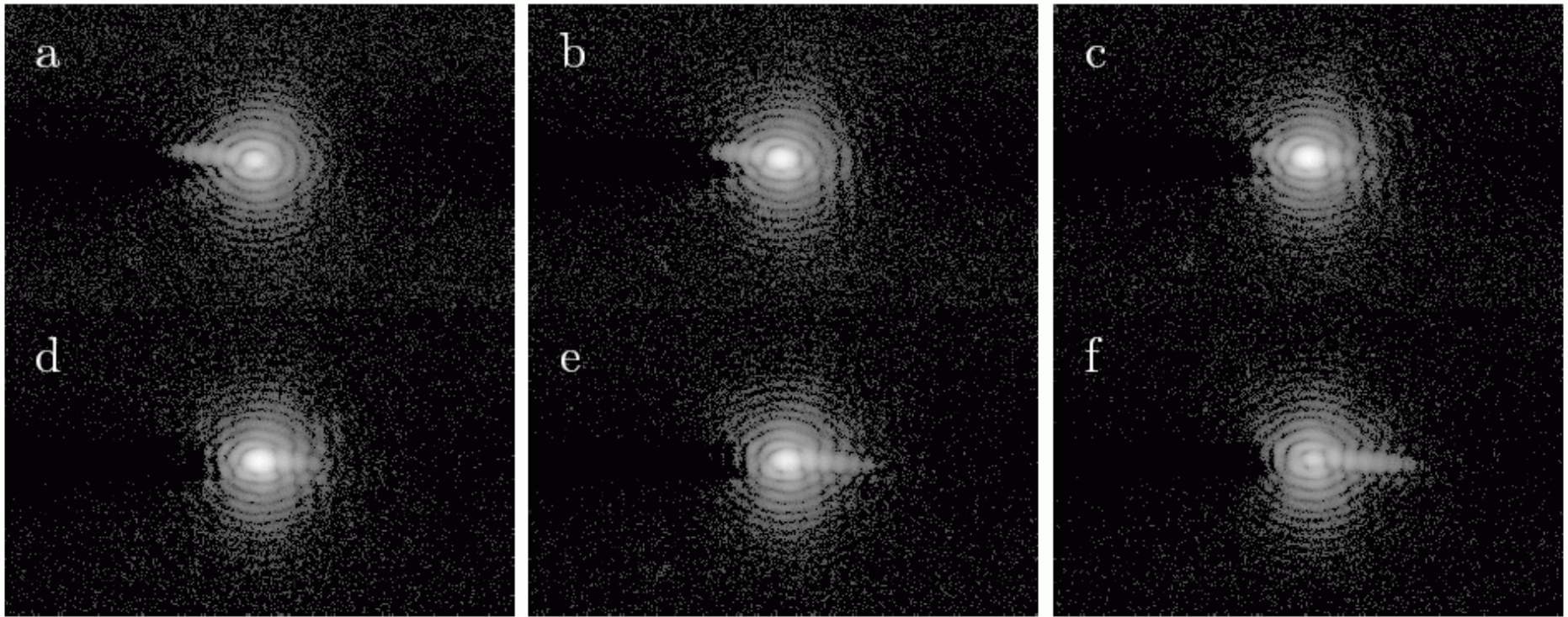


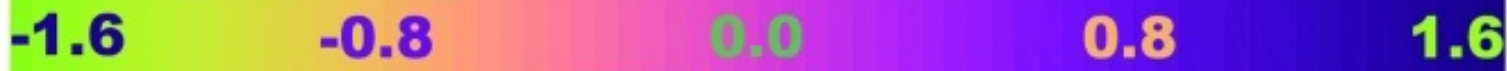
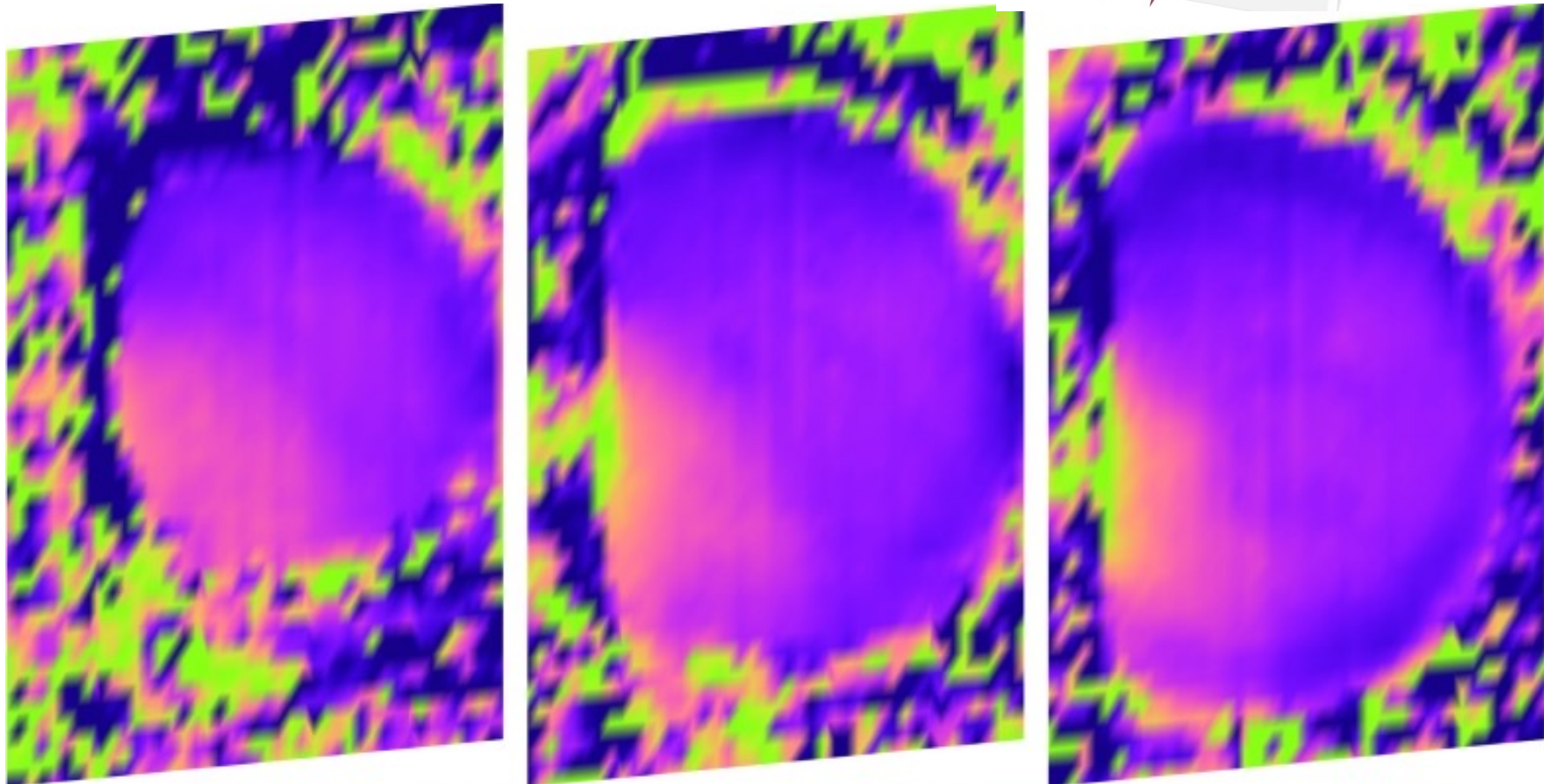
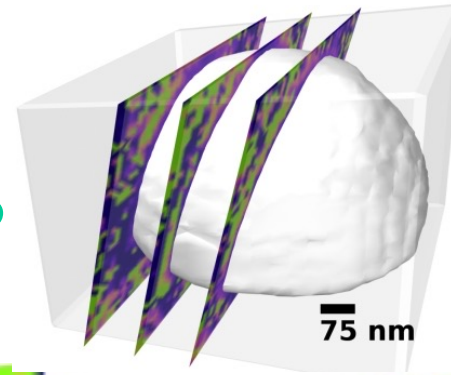
Figure 4.12: Center slices from 3D CXD pattern from Pb sample, on a log scale. Data file 296 from 10/03.

Facets of Equilibrium Crystal Shape

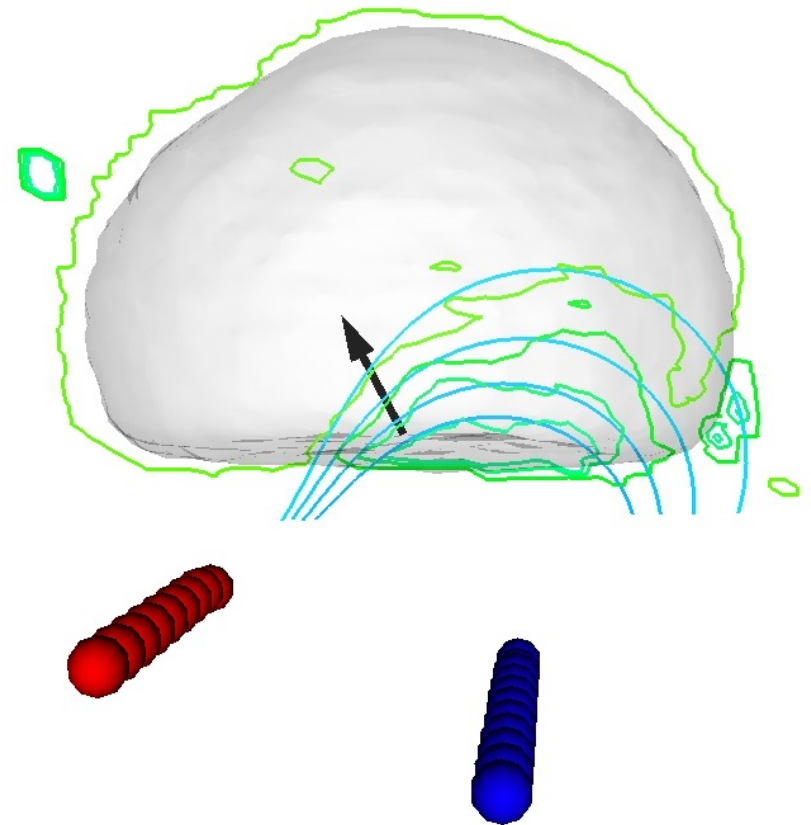
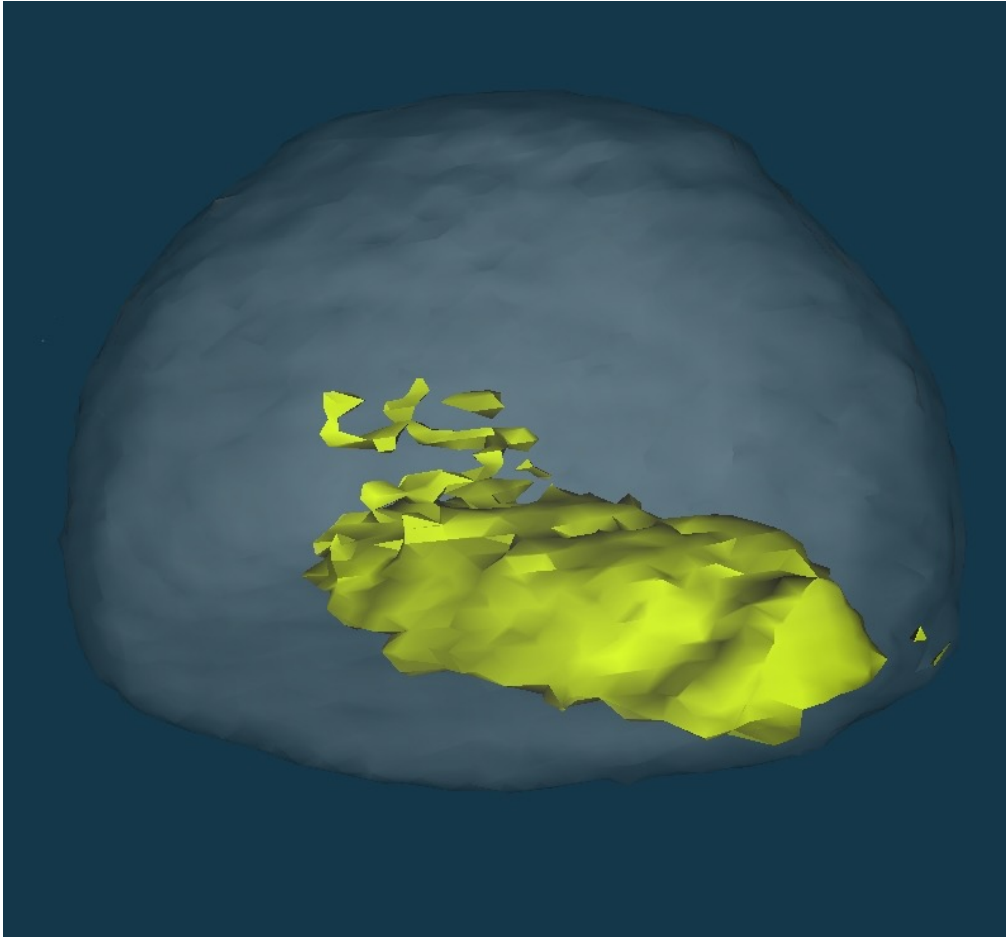


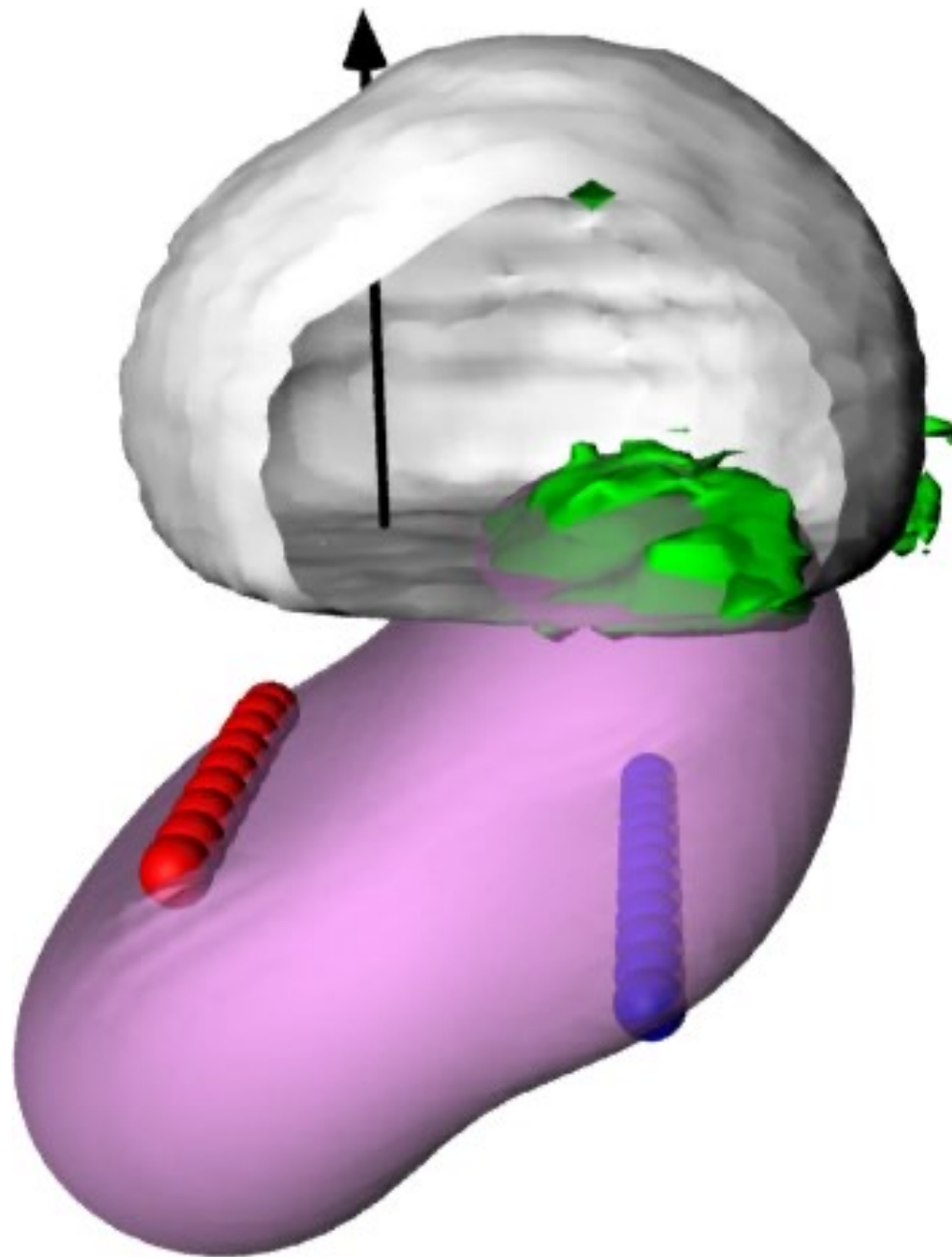
I. K. Robinson Astbury 2007

3D phase map sections



Modeling of 3D Phase Bump



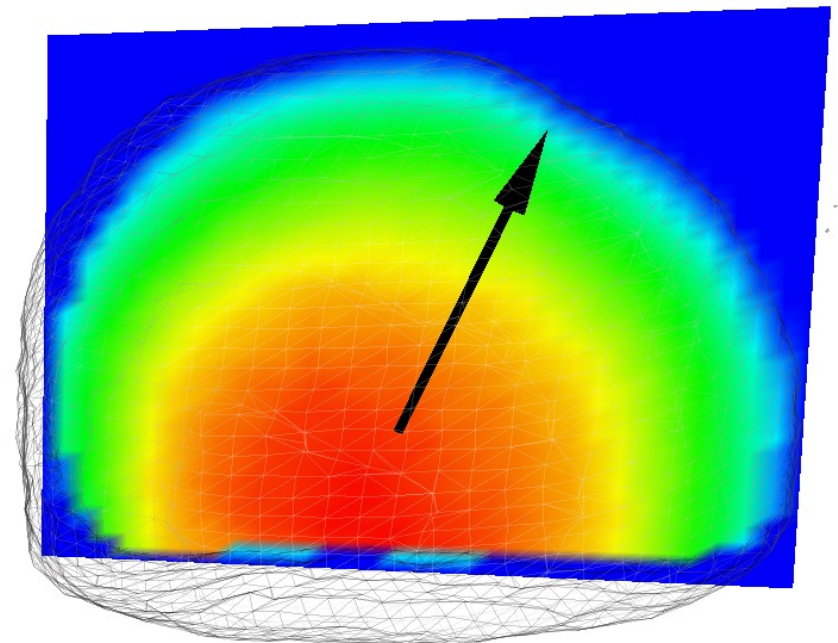
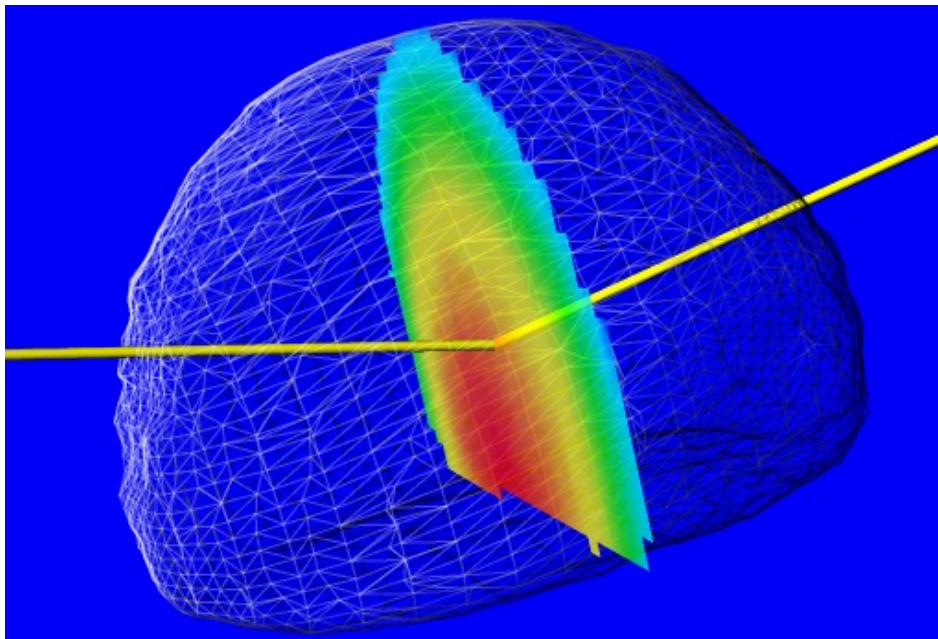


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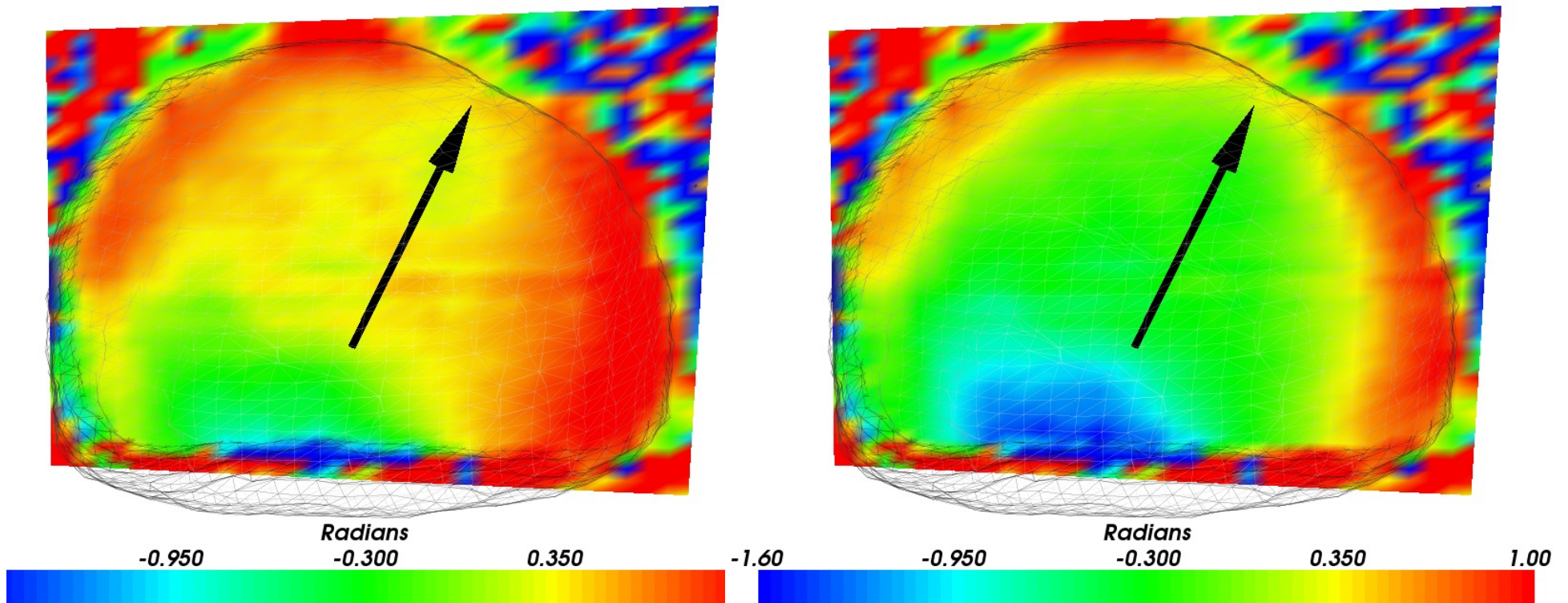
Refraction effects in Lead at 8.9keV

Phase accumulation due to refraction along scattering path
 $d=750\text{nm}$: $kd\delta = 0.76\text{rad}$
 $kd\beta = 0.07$

$$\delta = 2.23 \times 10^{-5}$$
$$\beta = 2.19 \times 10^{-6}$$

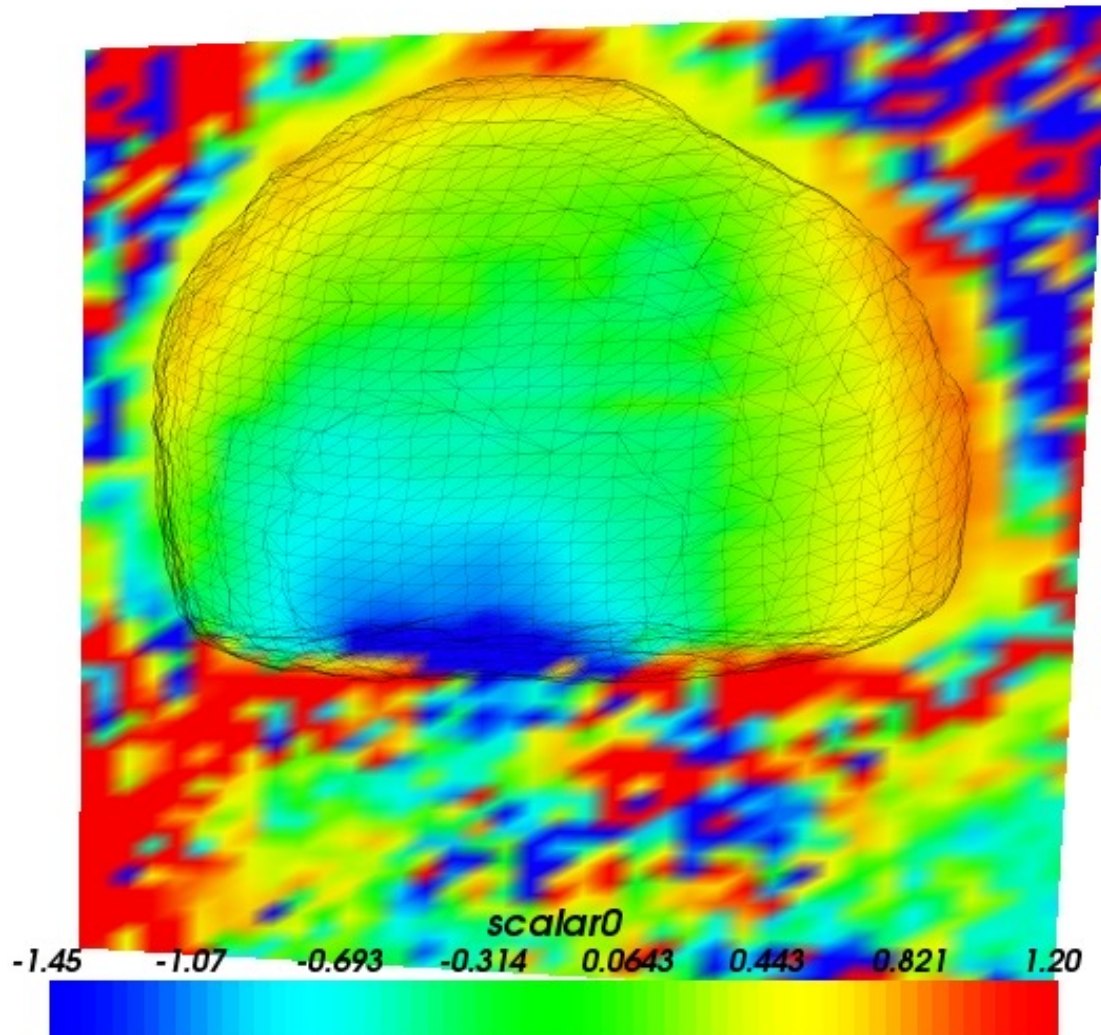


Phase Maps before and after Correction



Positive Phase is Surface Expansion

including correction for refraction by crystal



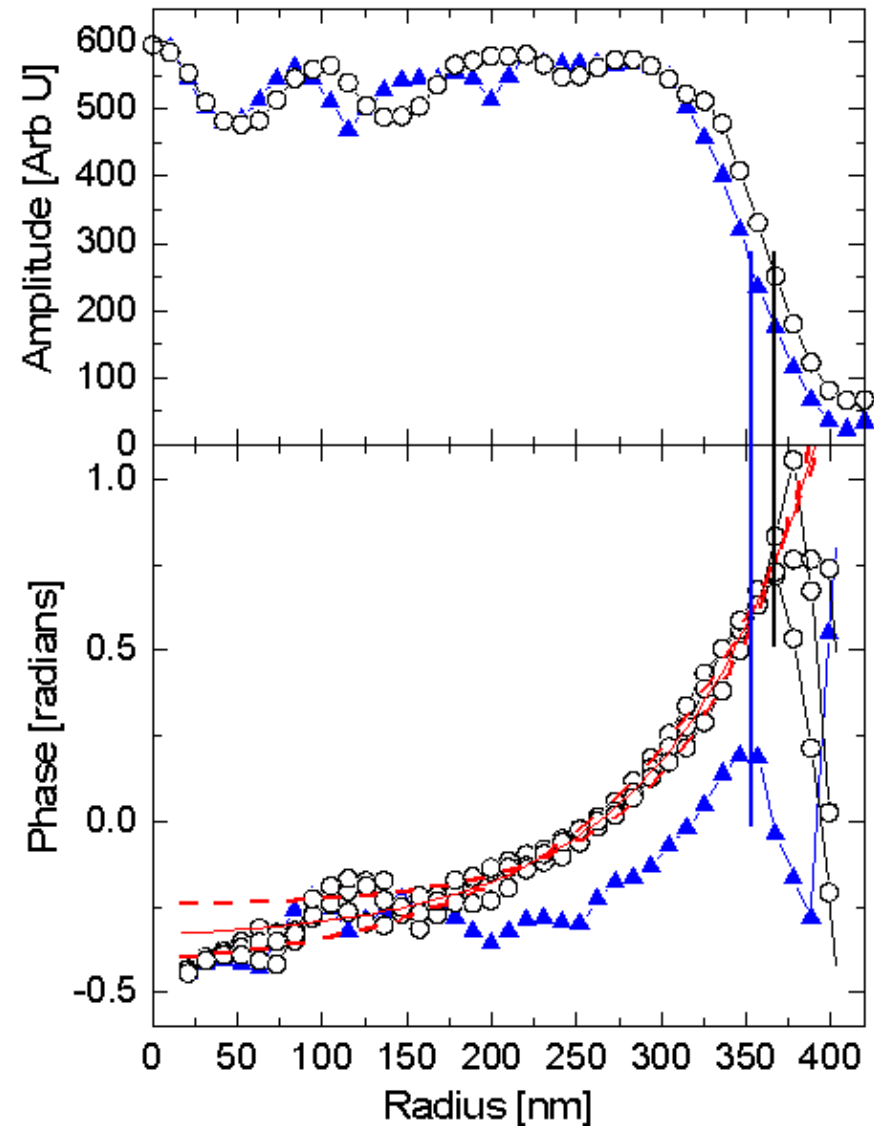
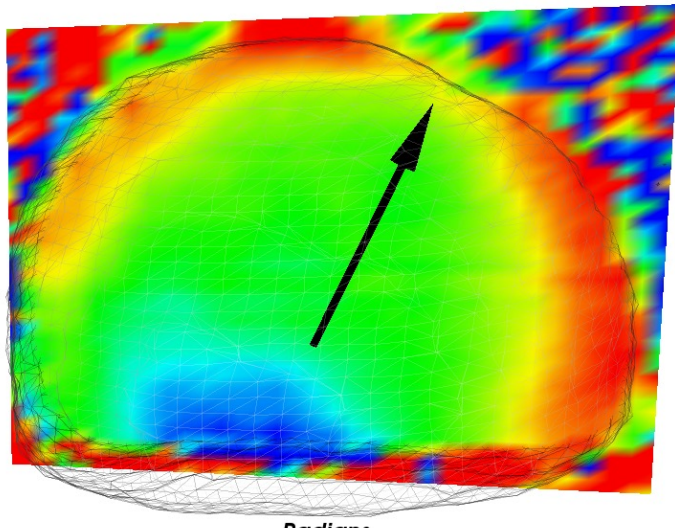
Max phase = 1.15rad
= 0.052nm

Phase on (111) facet:
= 0.47 rad
= 0.02nm

Depth Variation of Distortion

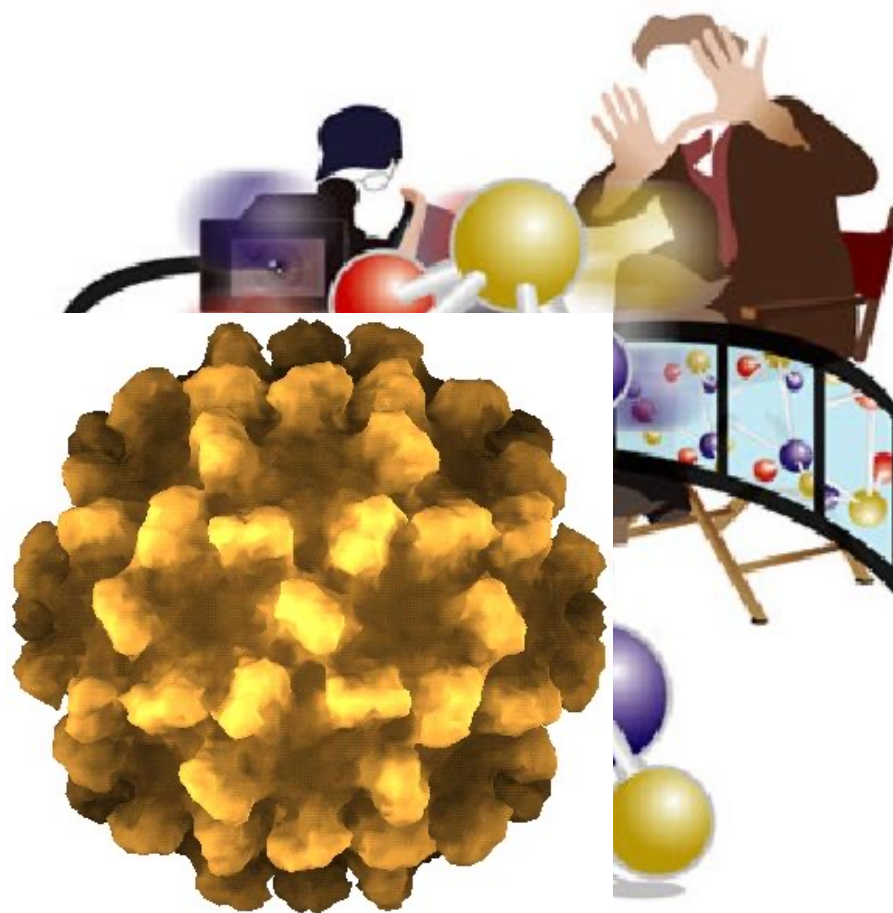
Facets subtend $18.6 \pm 2.6^\circ$
ECS predicts 14°

Depth measured $90 \pm 20\text{nm}$
Theory says 75nm



I. K. Robir

Molecular Movies using XFEL



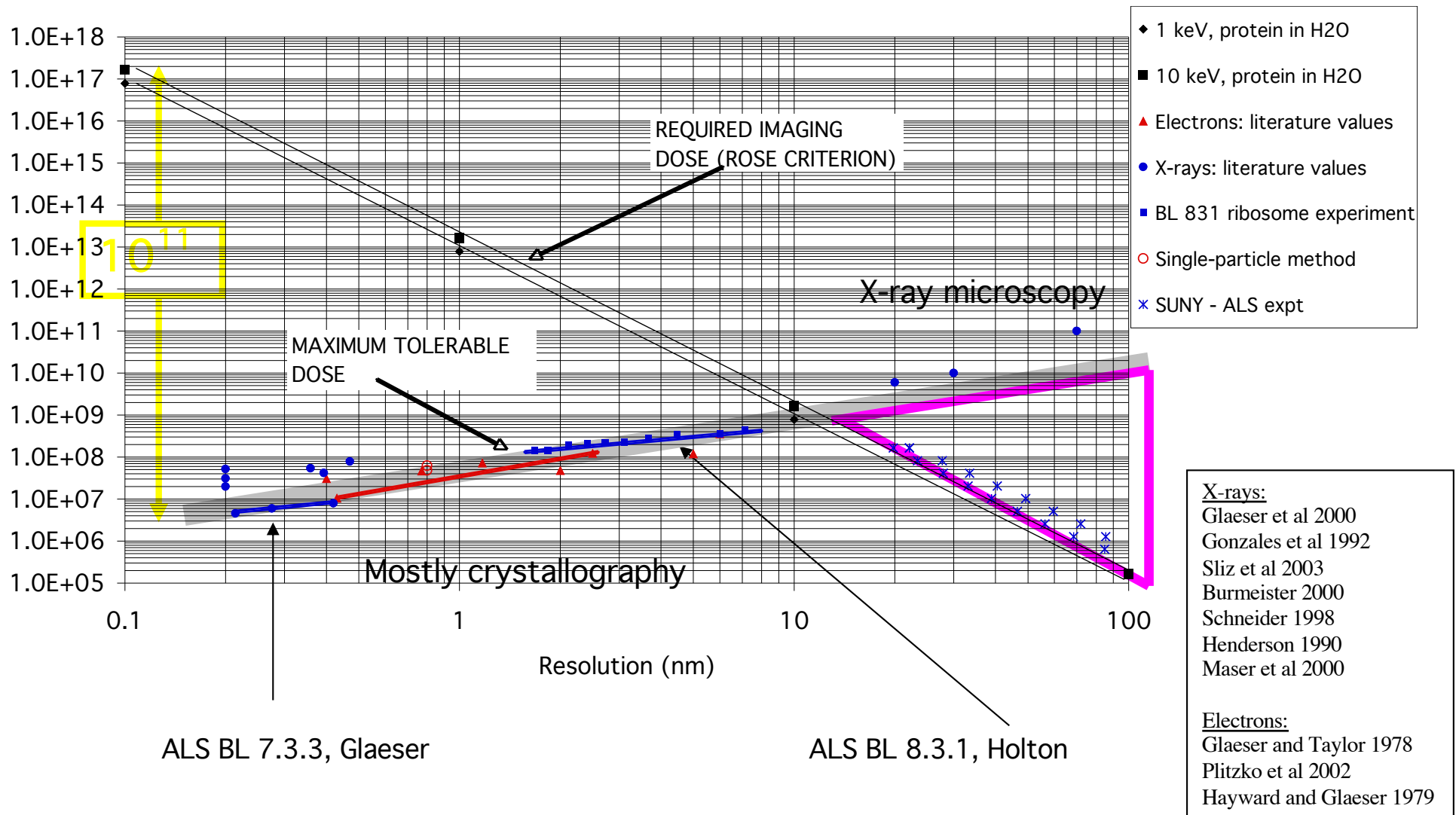
I. K. Robinson Astbury 2007

New routes to structure?

- Cryo-EM
 - real-space image, but not dose-efficient
- Inversion of diffraction
 - dose-efficient, especially for phase contrast
 - about equally efficient for photons or electrons
- How to beat the dose limit?

Do it faster!

Dose-resolution Relationship For 3D Imaging Of Frozen-hydrated Samples



c/o Malcolm Howells, ALS

Astbury 2007

How fast must we go?

- Radiation breaks bonds and ionizes matter
- Inertial response of nucleus to broken bond
- Structure is 'lost' when an atom moves by one bond-length
- Phonon vibration time: 10^{14} Hz = 10fs
- Photons better than electrons (space charge)
- Possible with X-ray free-electron laser (XFEL)

Conclusions

- Smallest Ferritin crystals about 3 microns
- Implosion due to radiation damage
- Expanded aggregate state upon freezing
- Model SAXS using clusters
- Single molecule diffraction ideas can be tested using nanocrystals
- Phase objects 'equally' accessible
- Come to Diamond!