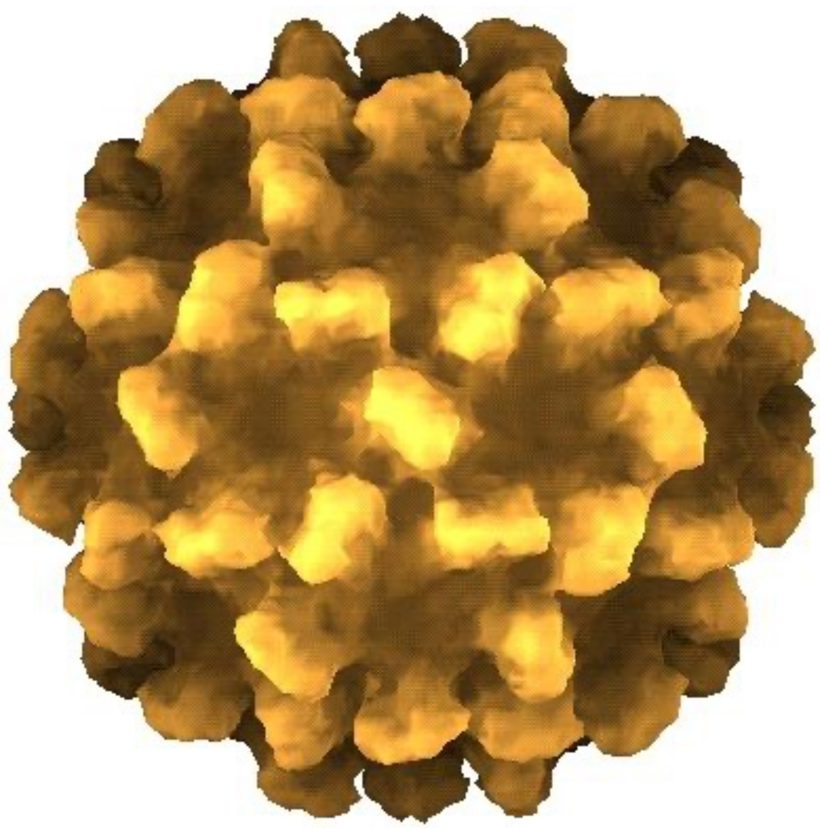
Coherent and Solution Scattering investigation of the Nucleation of Protein Crystals

- Ian Robinson
- Sébastien Boutet
- Franz Pfeiffer
- Ivan Vartaniants
- 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)

Biology with Synchrotron Radiation,
BSR2007
Manchester UK

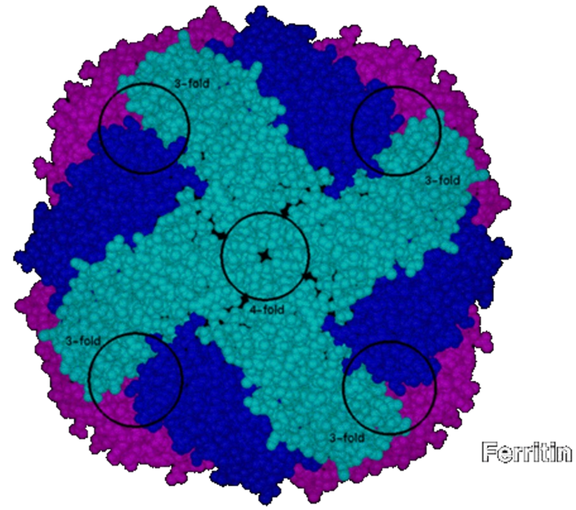
Tomato Bushy Stunt Virus
1980

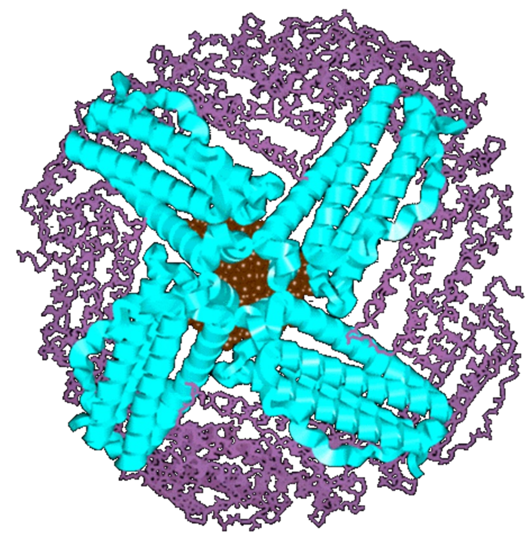


I. K. Robinson   BSR2007 Manchester

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 : Holoferritin
- Empty Shell : Apoferritin





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.

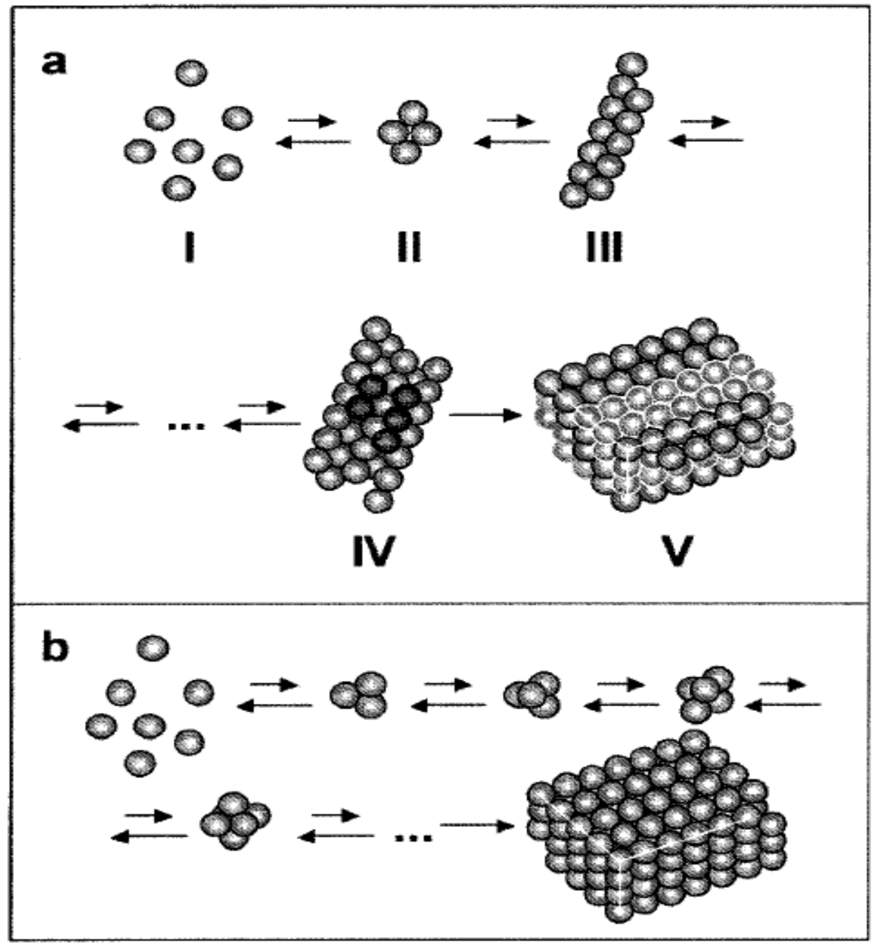
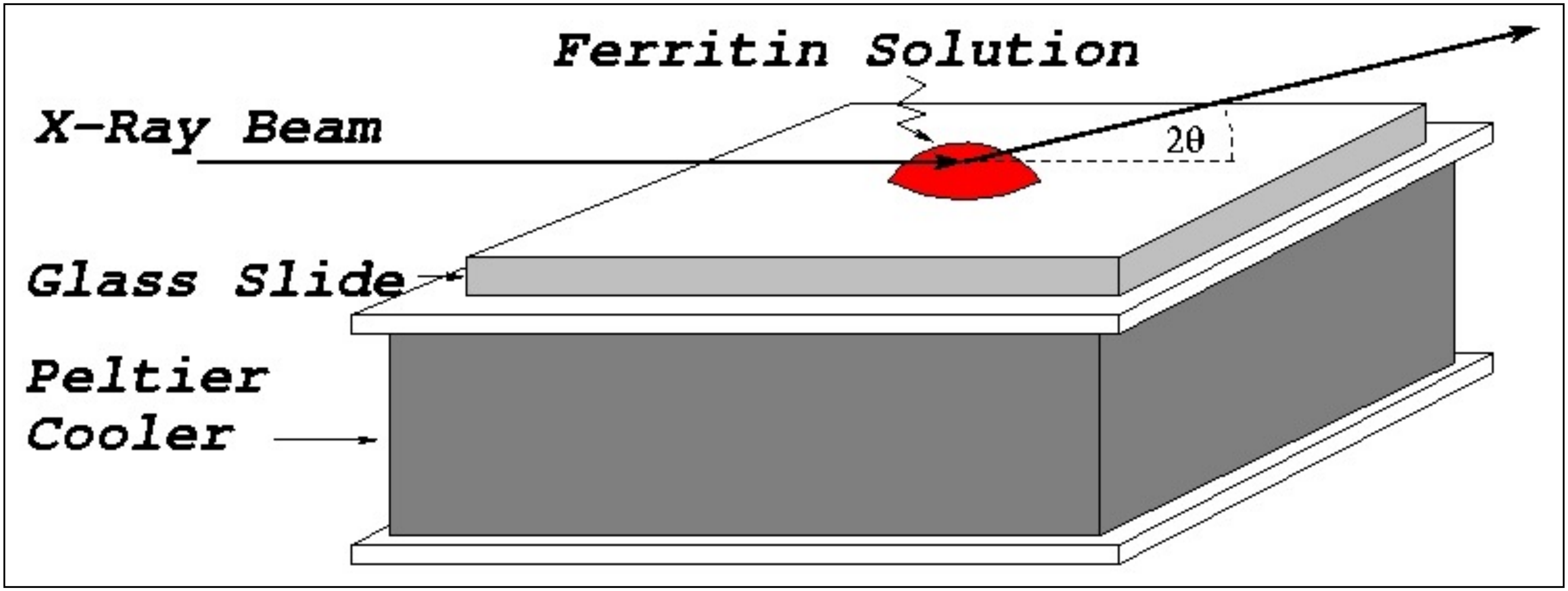
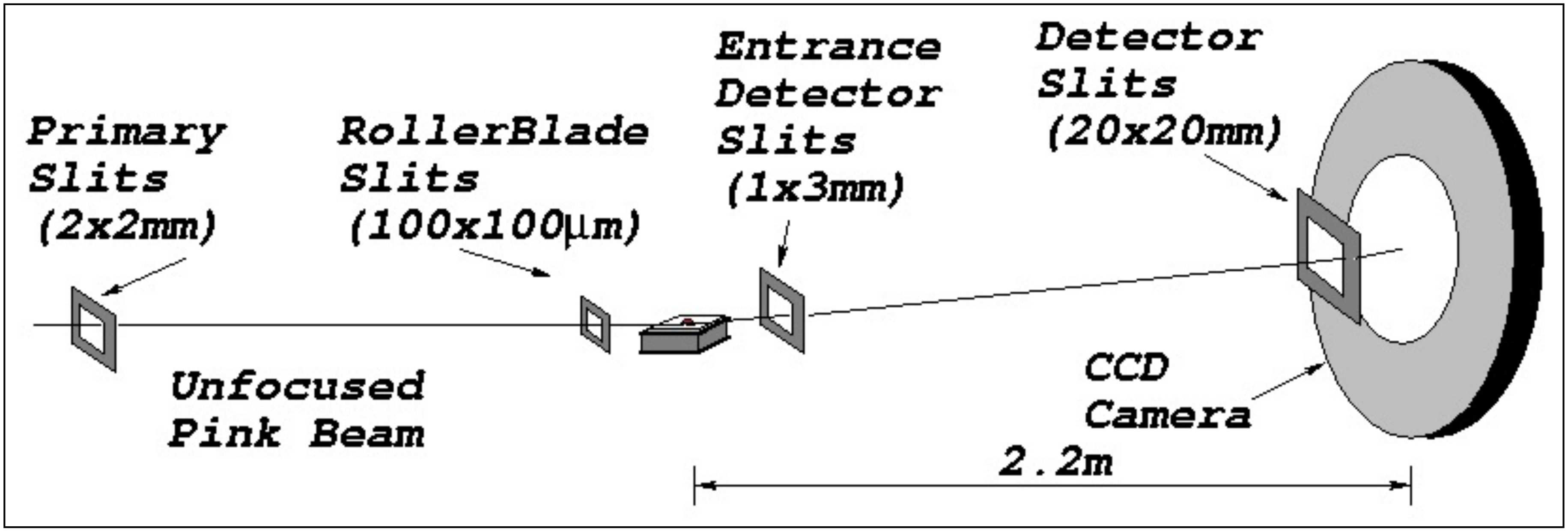


Figure 10. Schematic illustration of two nucleation pathways: (a) via a planar critical cluster (in IV molecules belonging to the second layer are shown in a lighter shade; in V the (110) layers that stack up to form this crystal are delineated by lighter and darker contours) and (b) via compact critical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

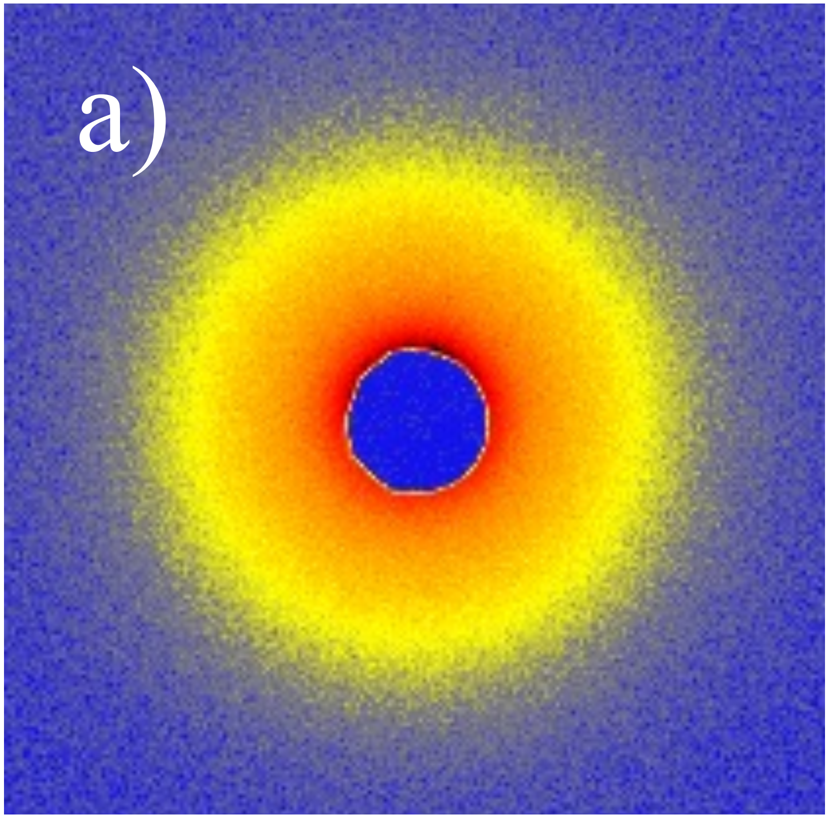
In-situ Study of Crystallization

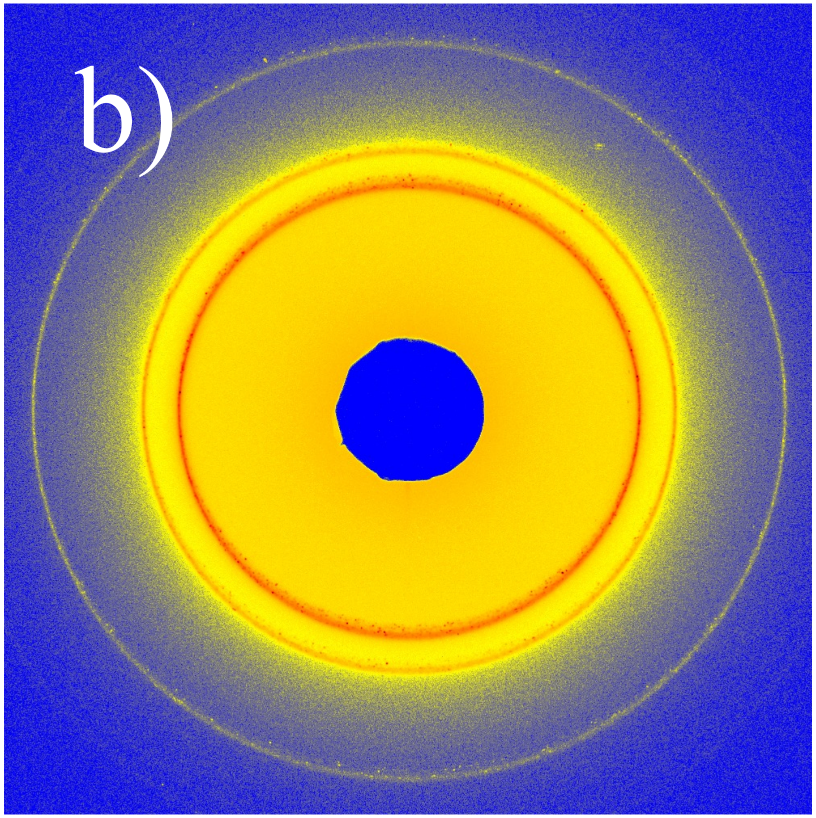


Experiment at APS Sector 34



Crystallization of Holoferritin: SAXS

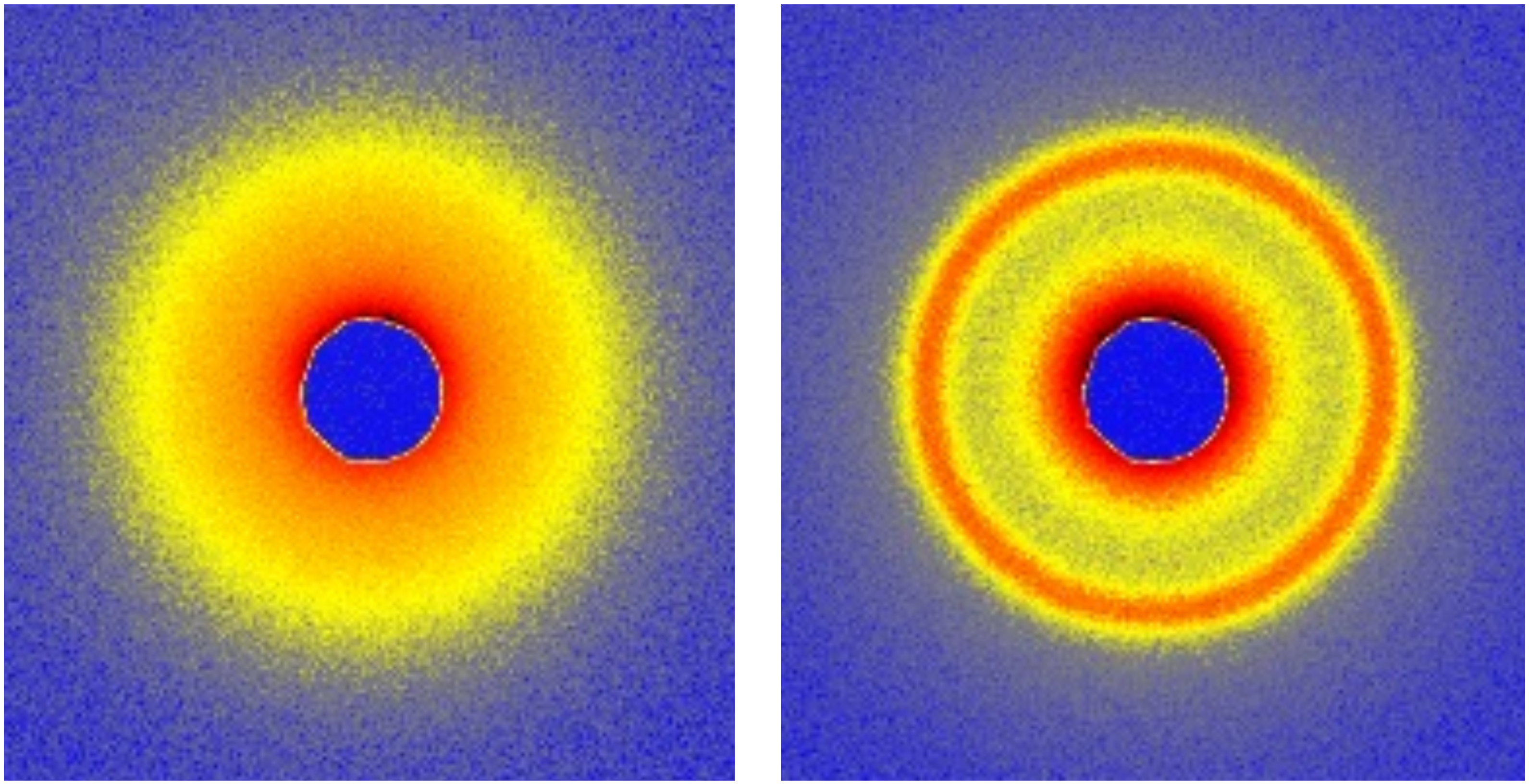




a) SAXS pattern of holoferritin at 10^{o}C.

b) SAXS pattern after adding Cadmium salt

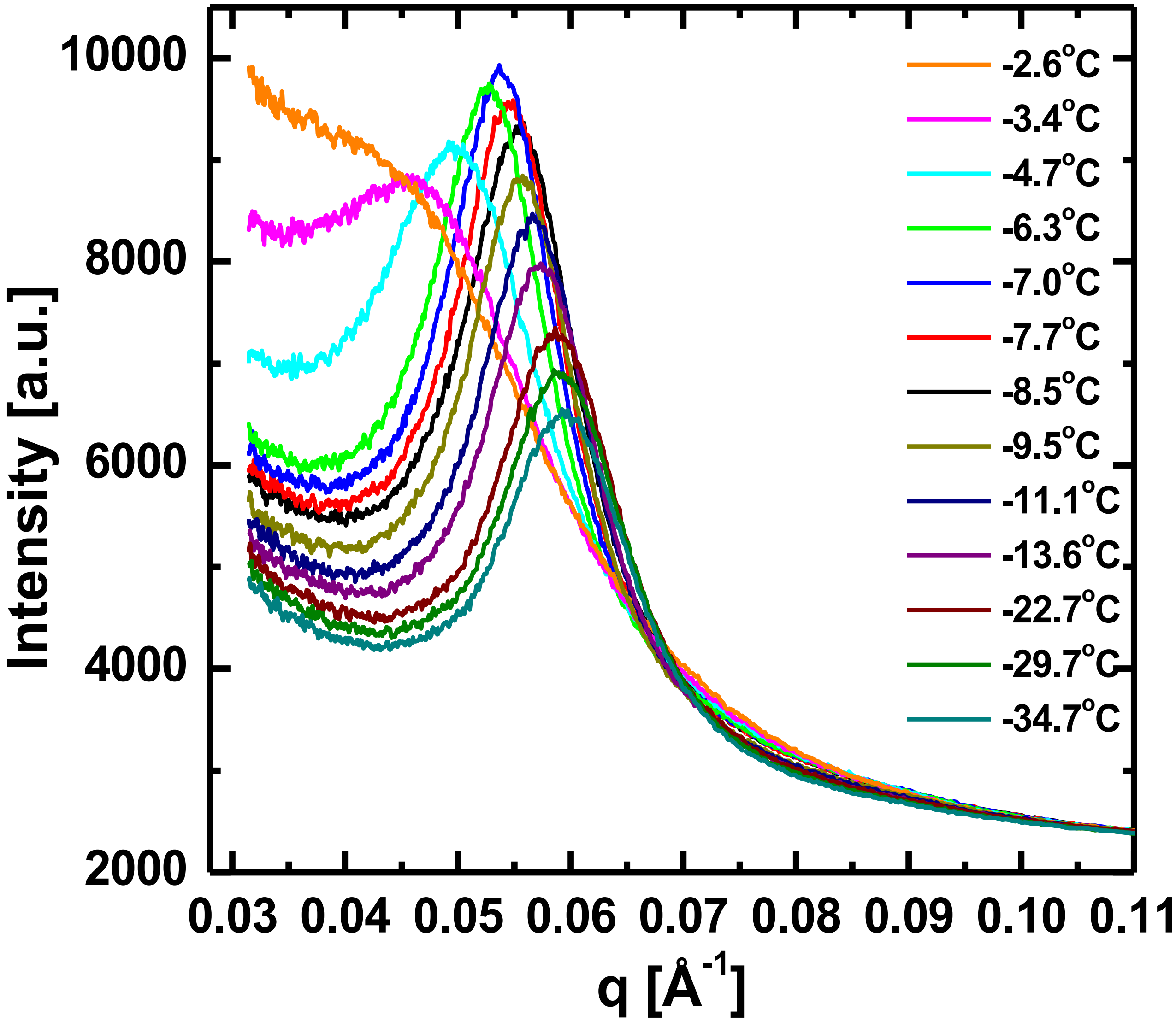
Effect of Temperature



SAXS pattern at T=+10^{o}C and -10^{o}C.

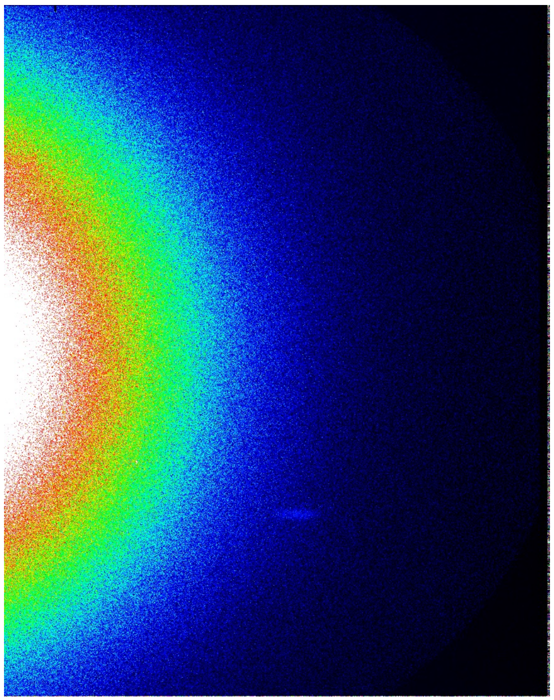
Holoferritin in dilute (10mg/ml) NaCl only.

Integrated SAXS Data

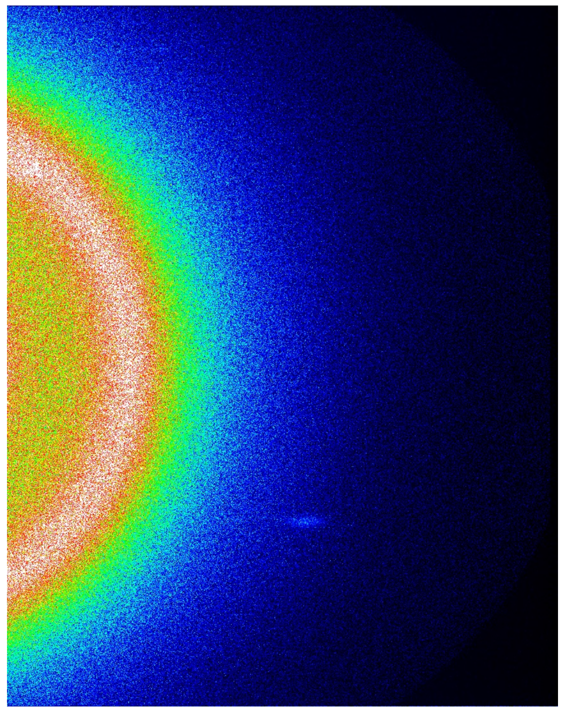


- The SAXS pattern shows a broad peak which shifts in position and changes in width as a function of temperature

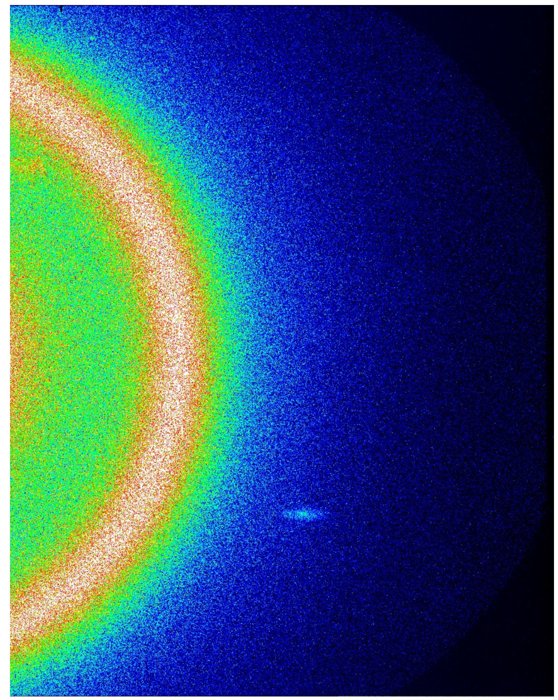
Ferritin Solution upon Freezing



T=13.3

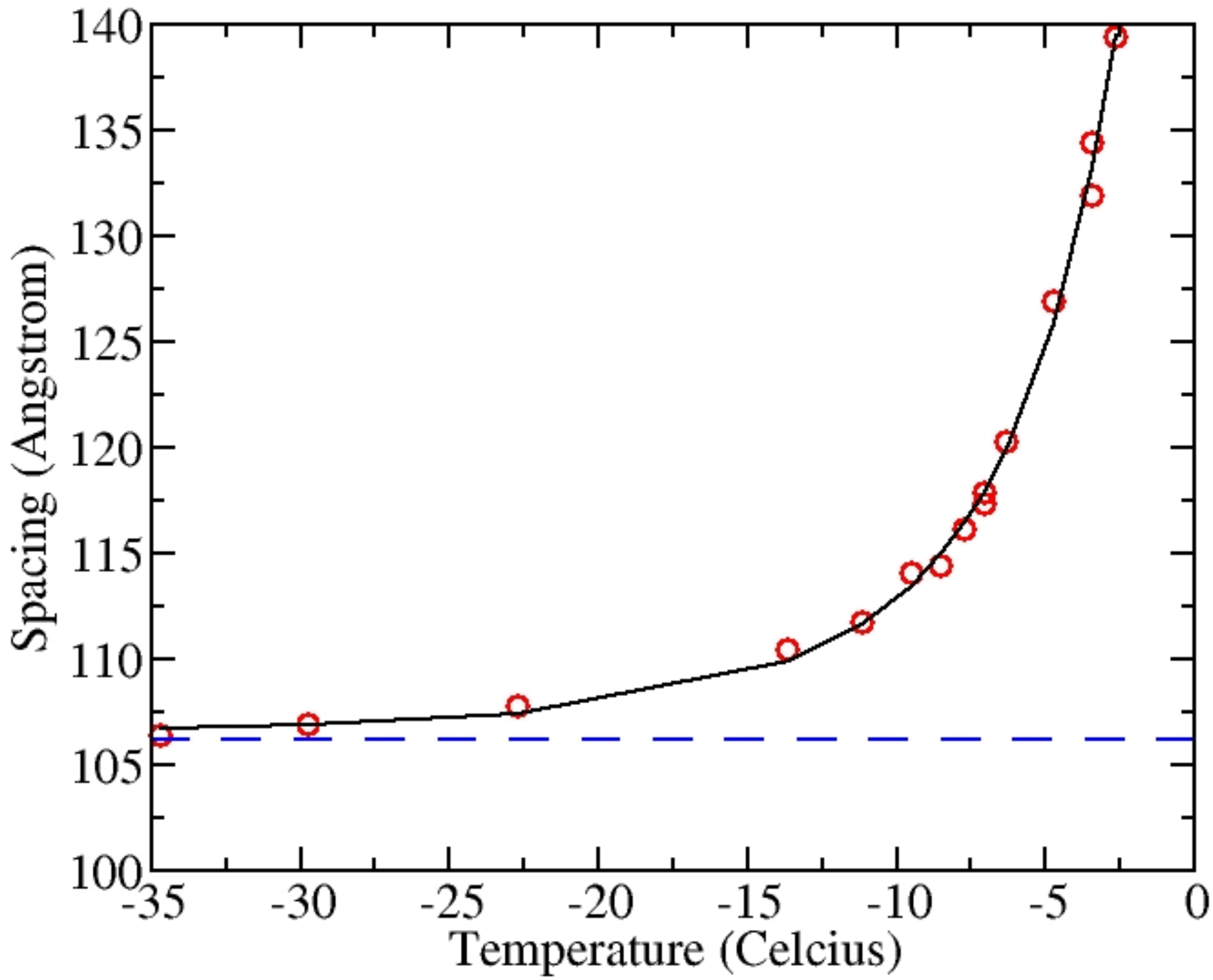


T=-4.7

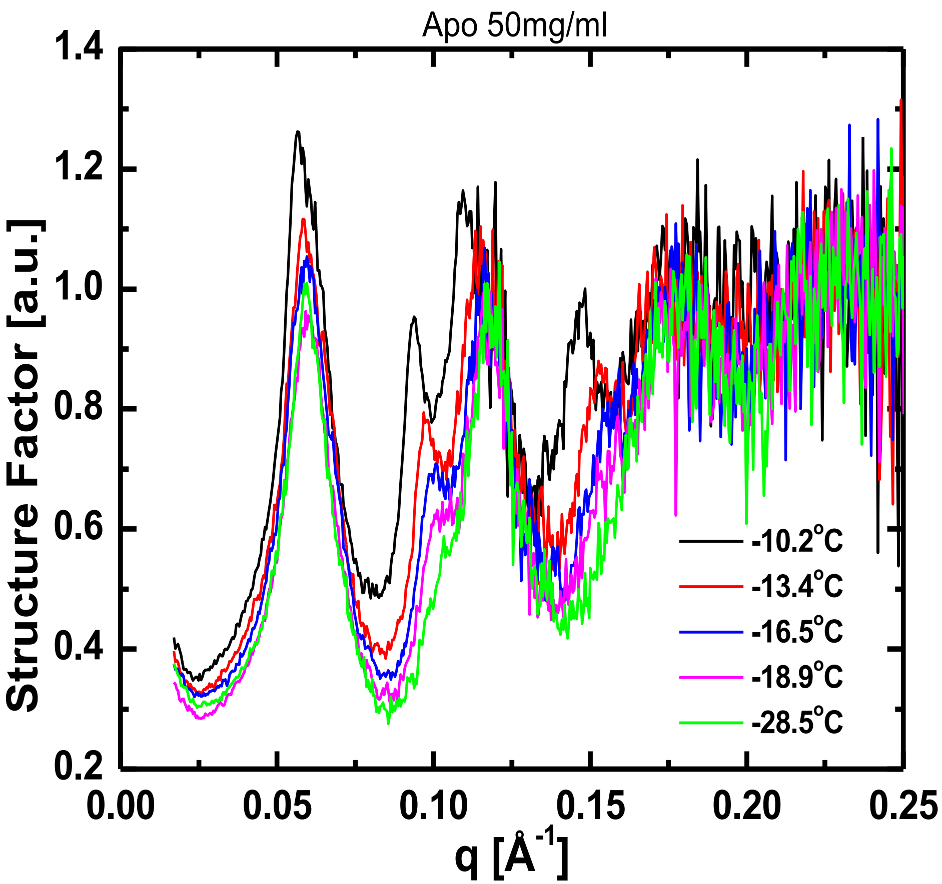


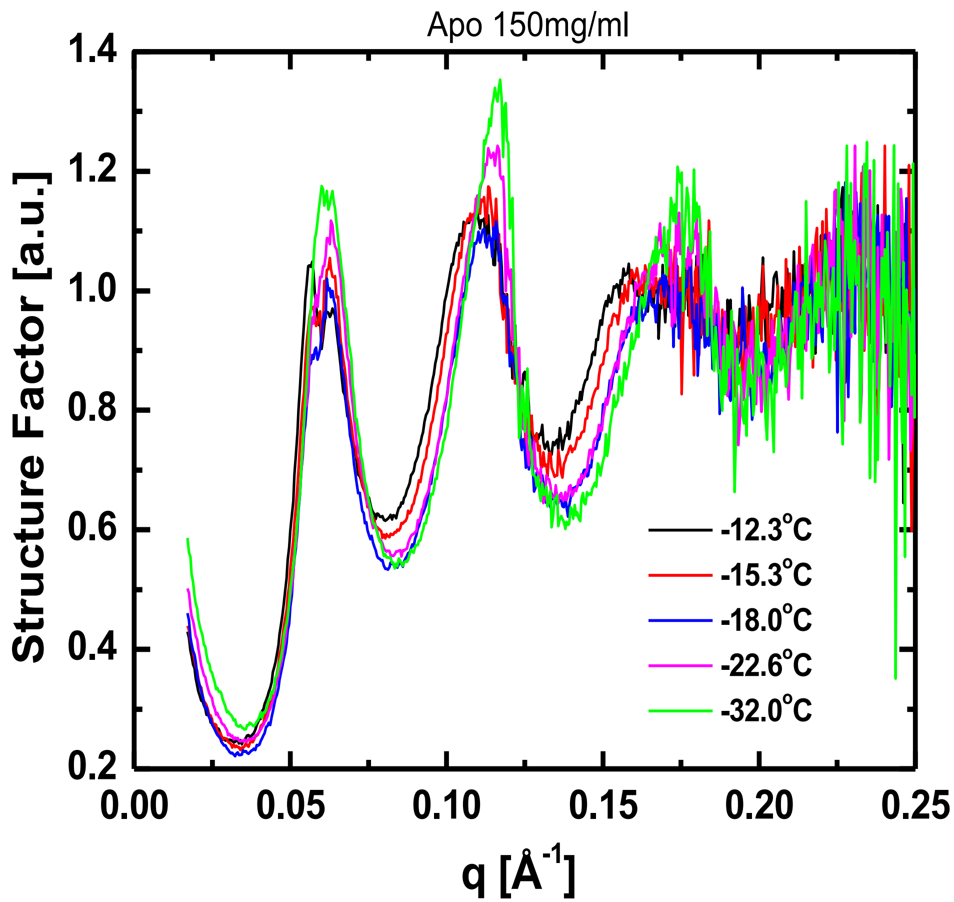
T=-34.7

Temperature Variation of Peak Position

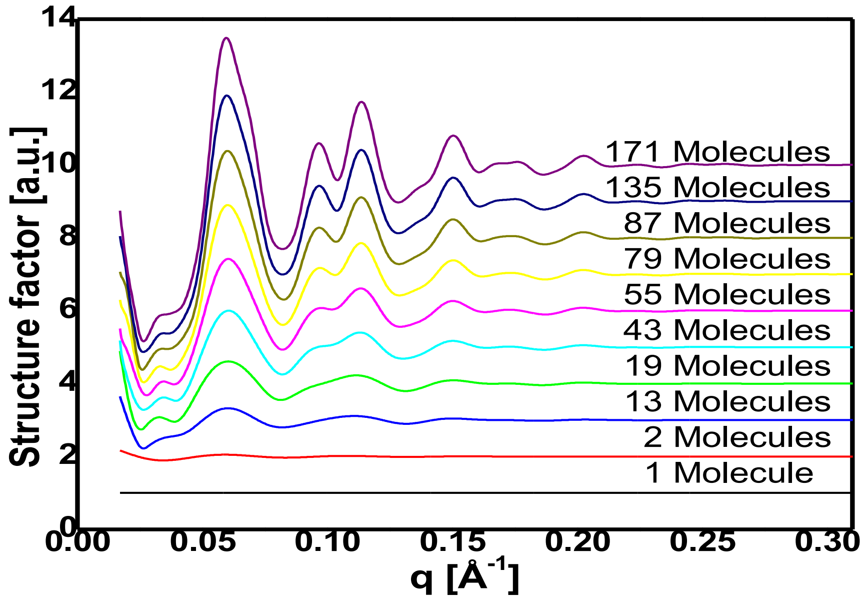


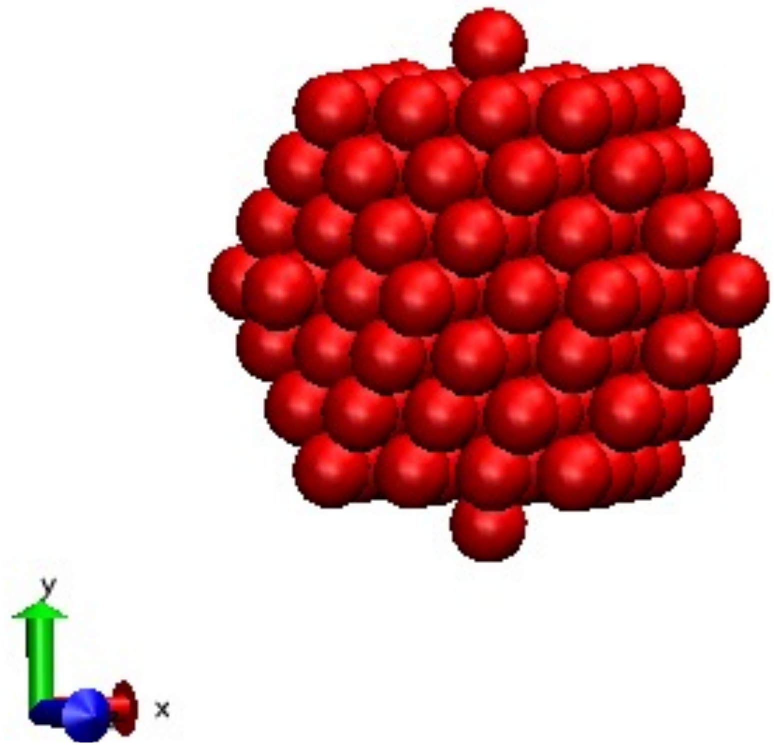
Structure Factors of Apoferritin





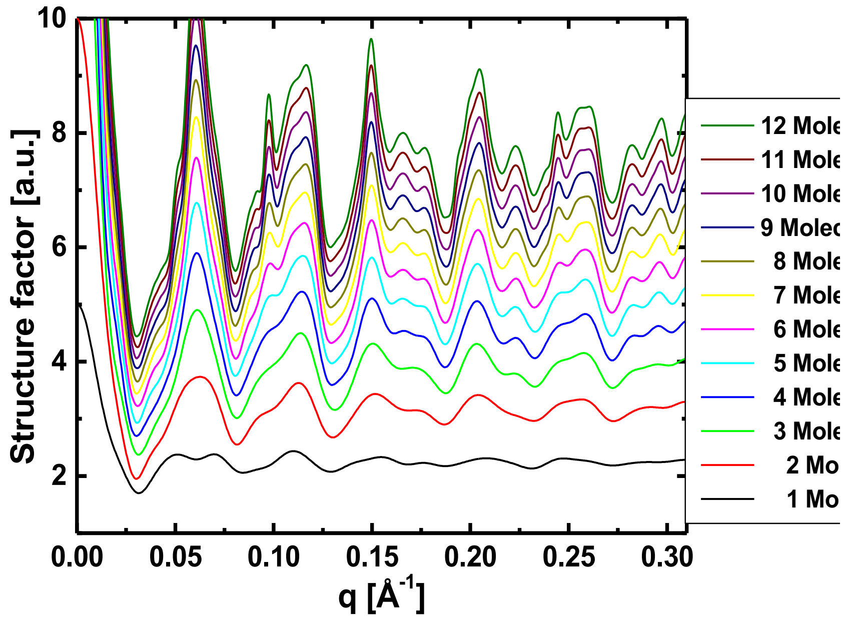
FCC Spherical Clusters

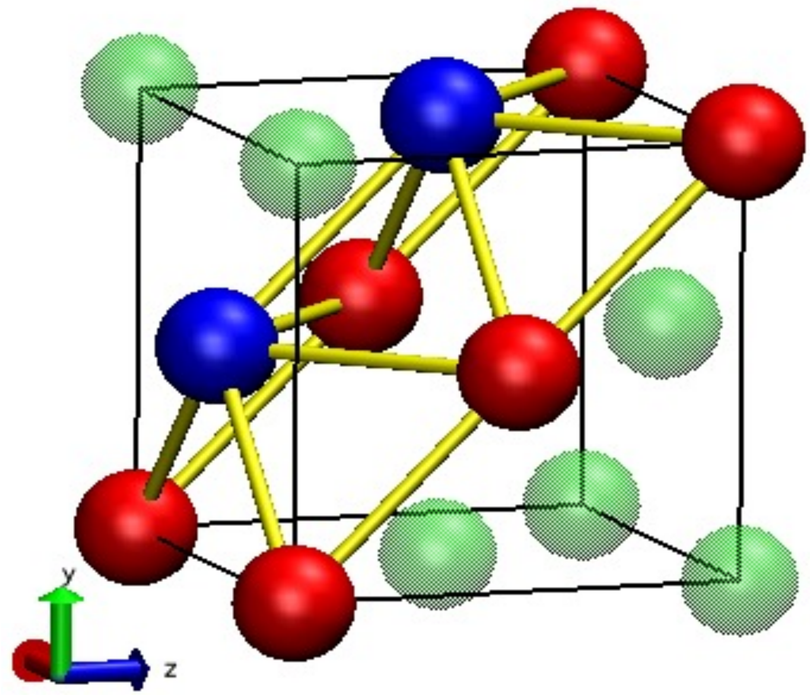




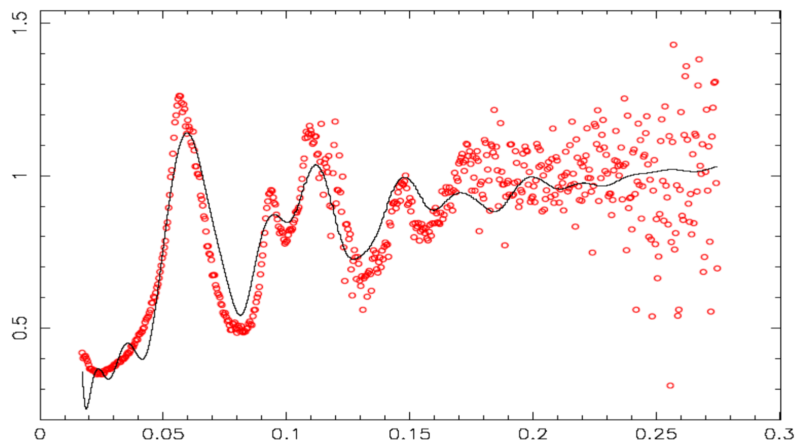
Planar Clusters of 110 Rods

Structure factor simulation as 5 <110> rods with 1 to 12 molecules per rod

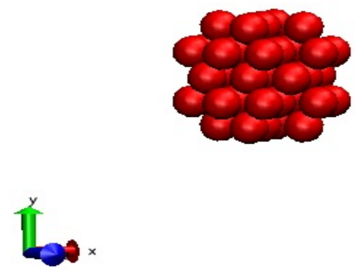


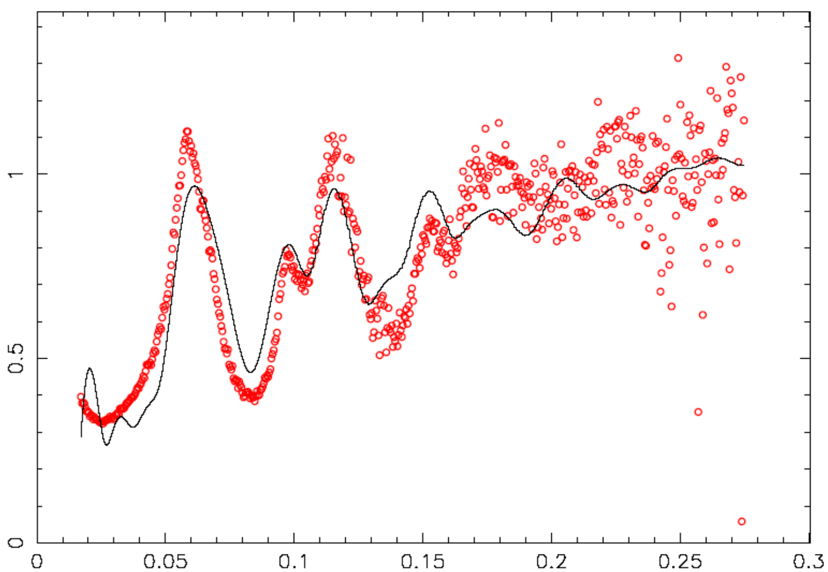


Best Fits

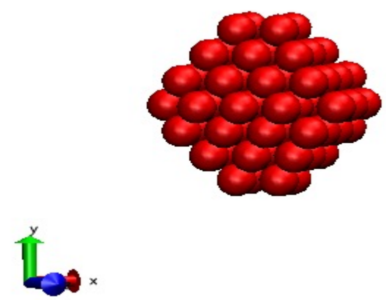


T=–10°C
Cluster Size = 36



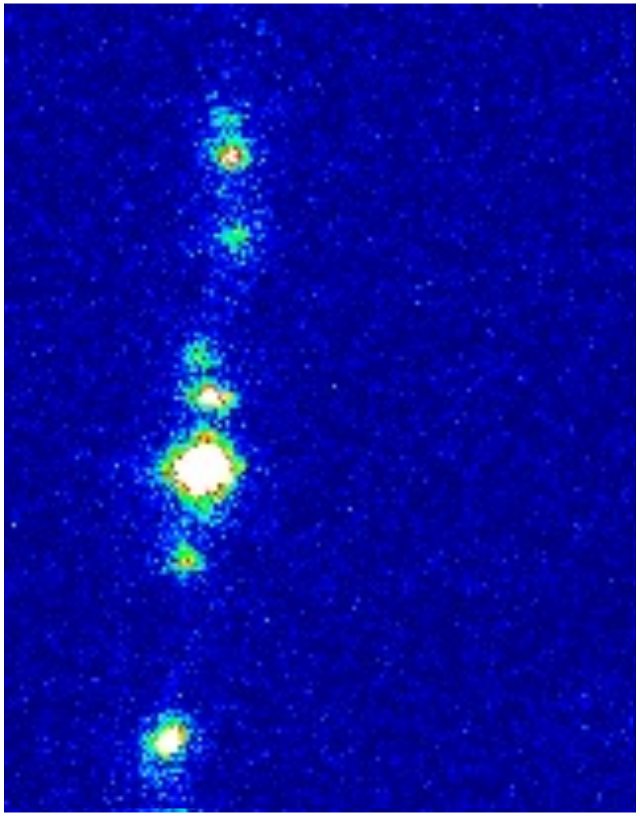


T=–13°C
Cluster Size = 63

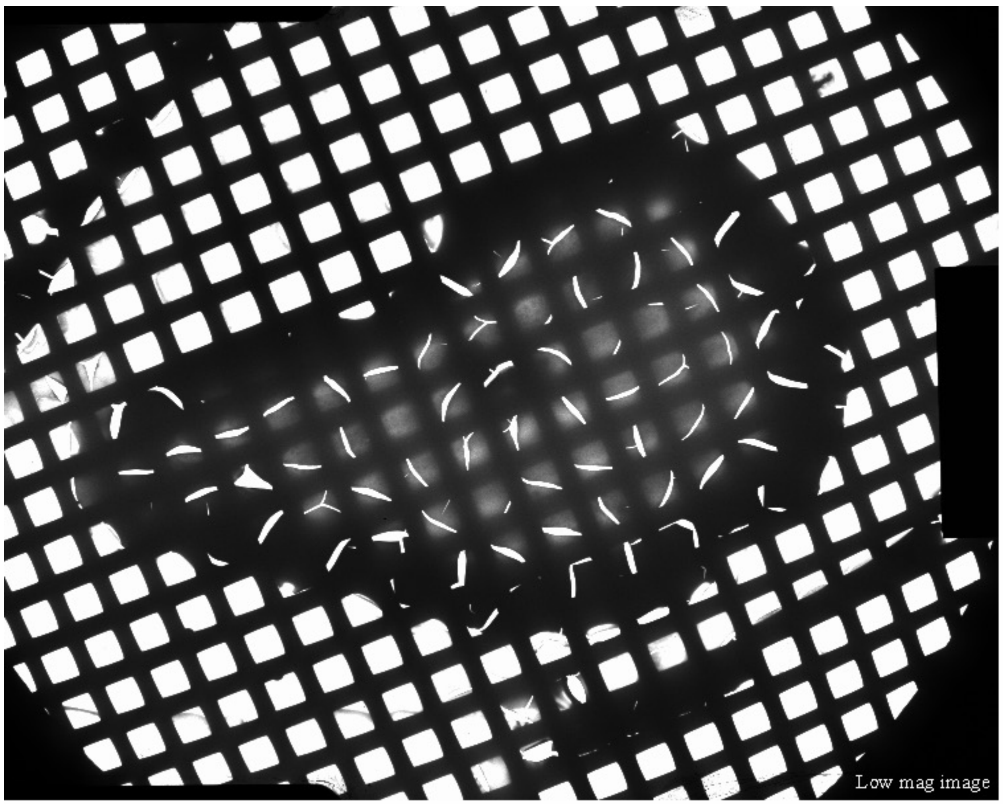


Ferritin (111) Powder Ring

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

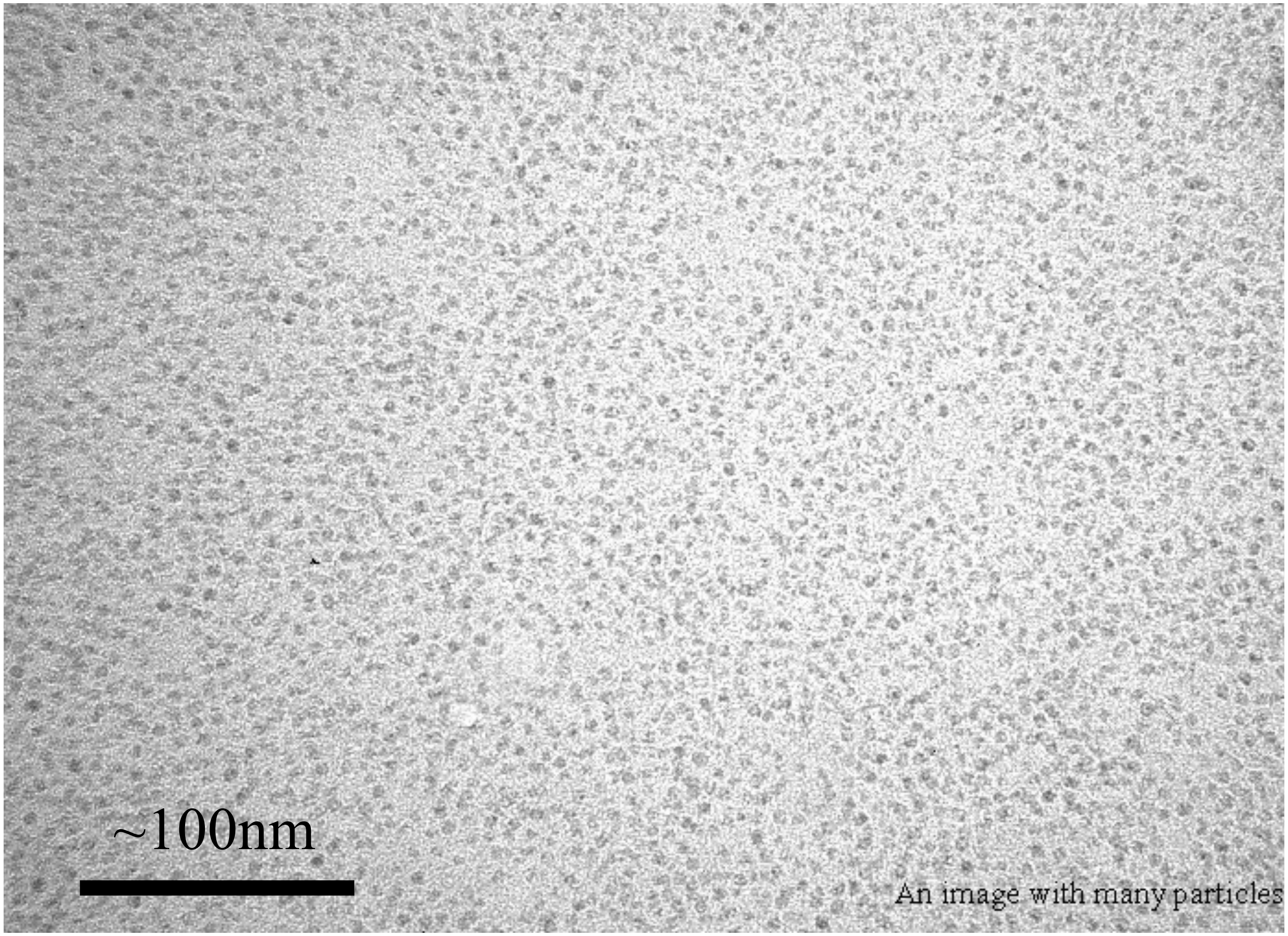


Overview of EM grid

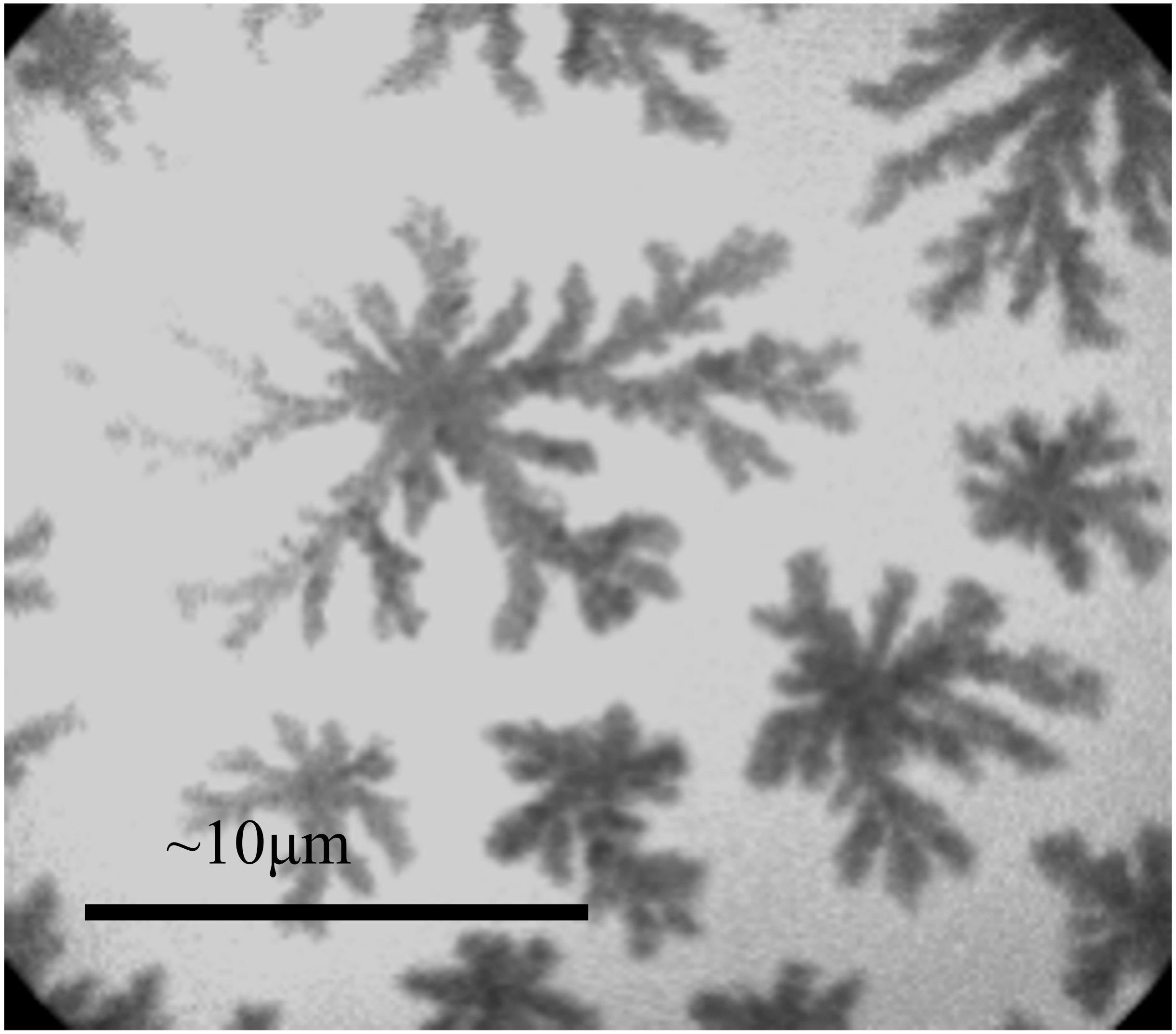


•3mm diam grid
•a-carbon + “Formvar”
•10mg/ml Ferritin
•10mg/ml NaCl
•dipped grid
•dried in air
•no cryo-cooling
  ▹three regions of different density

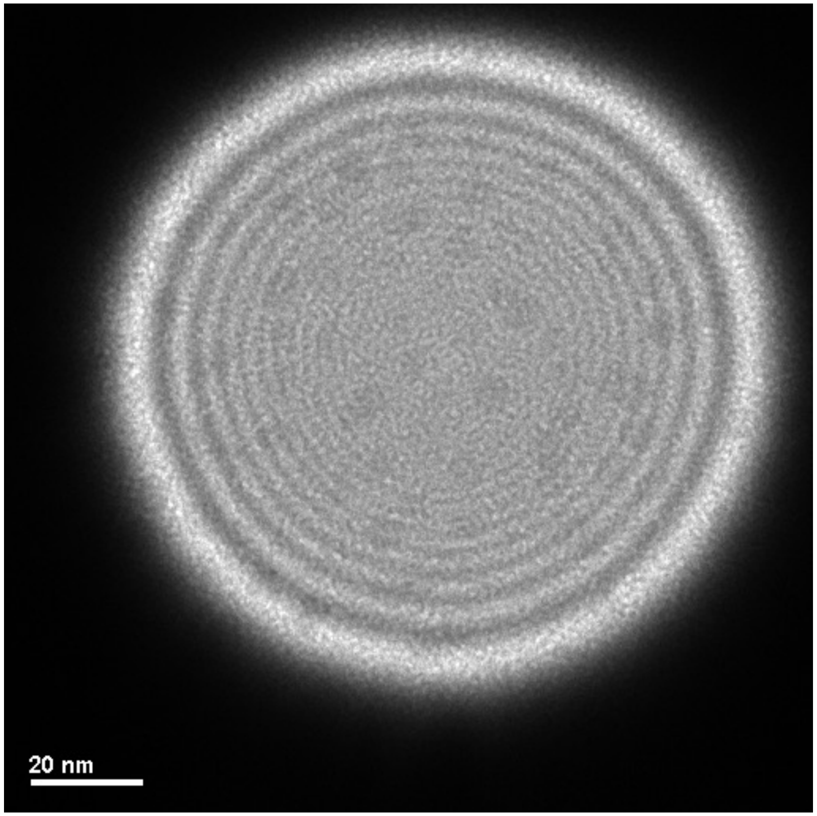
Border Region (low density)

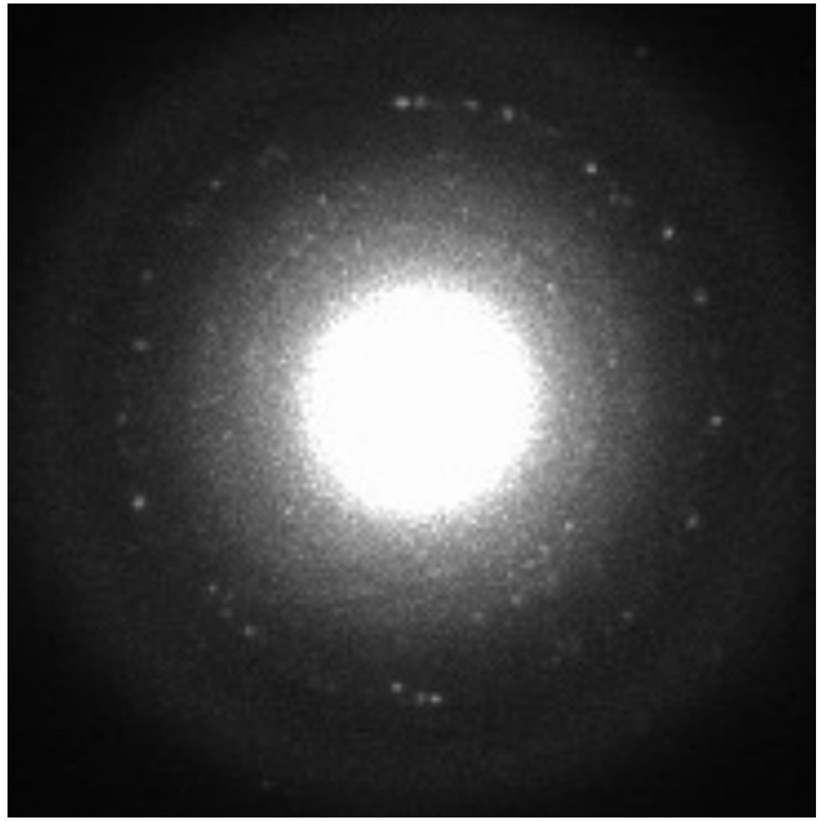


High Density Ring

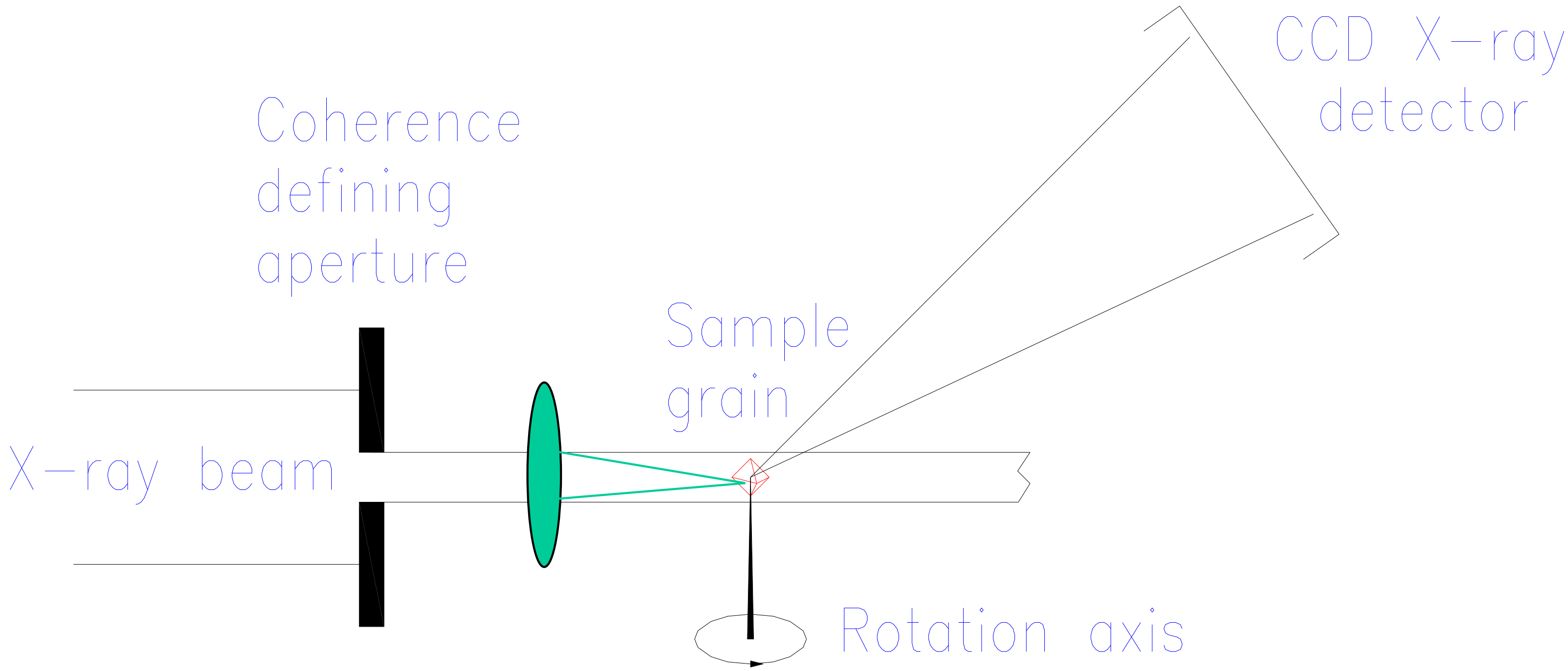


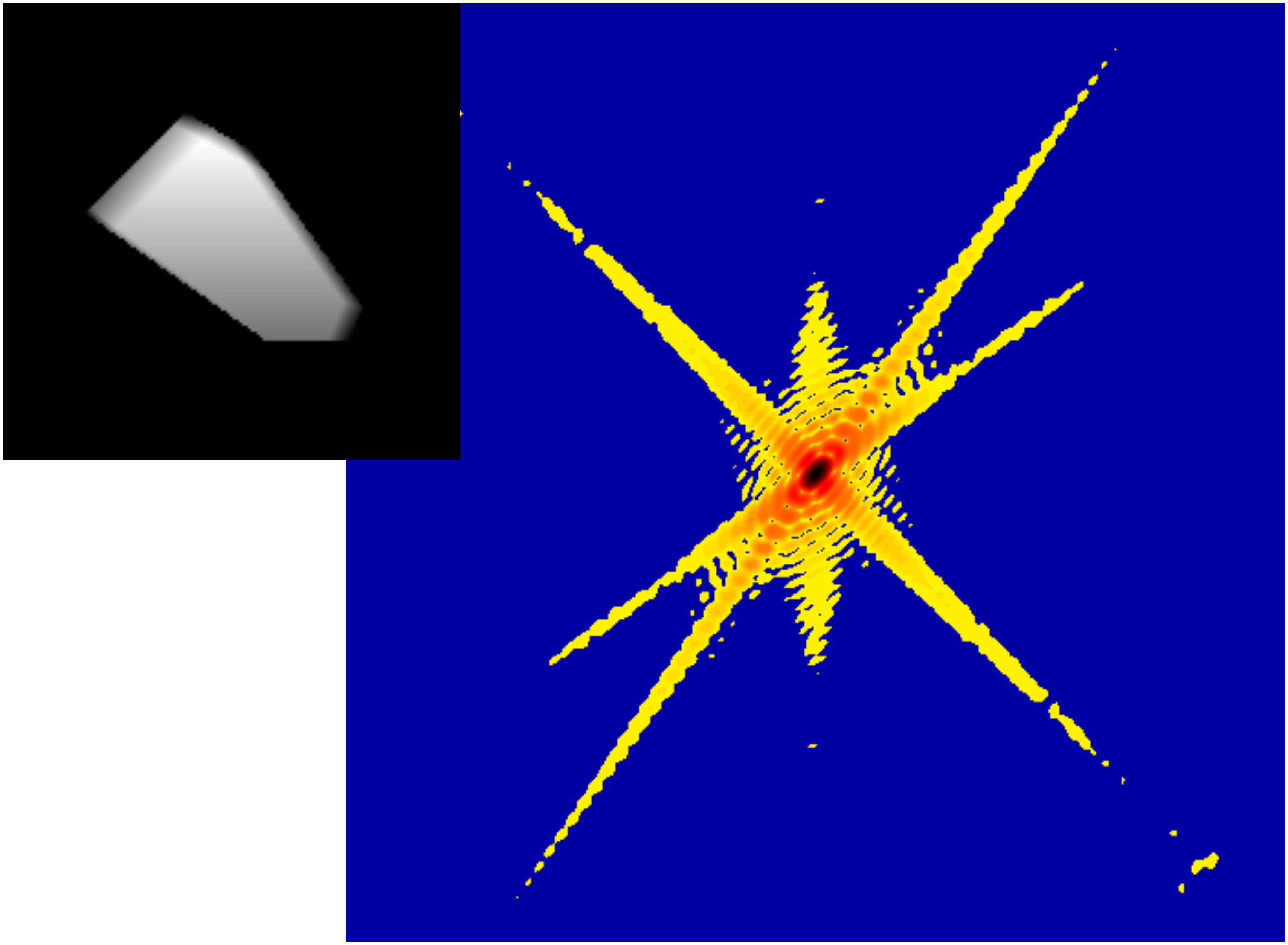
Diffraction from Border Region



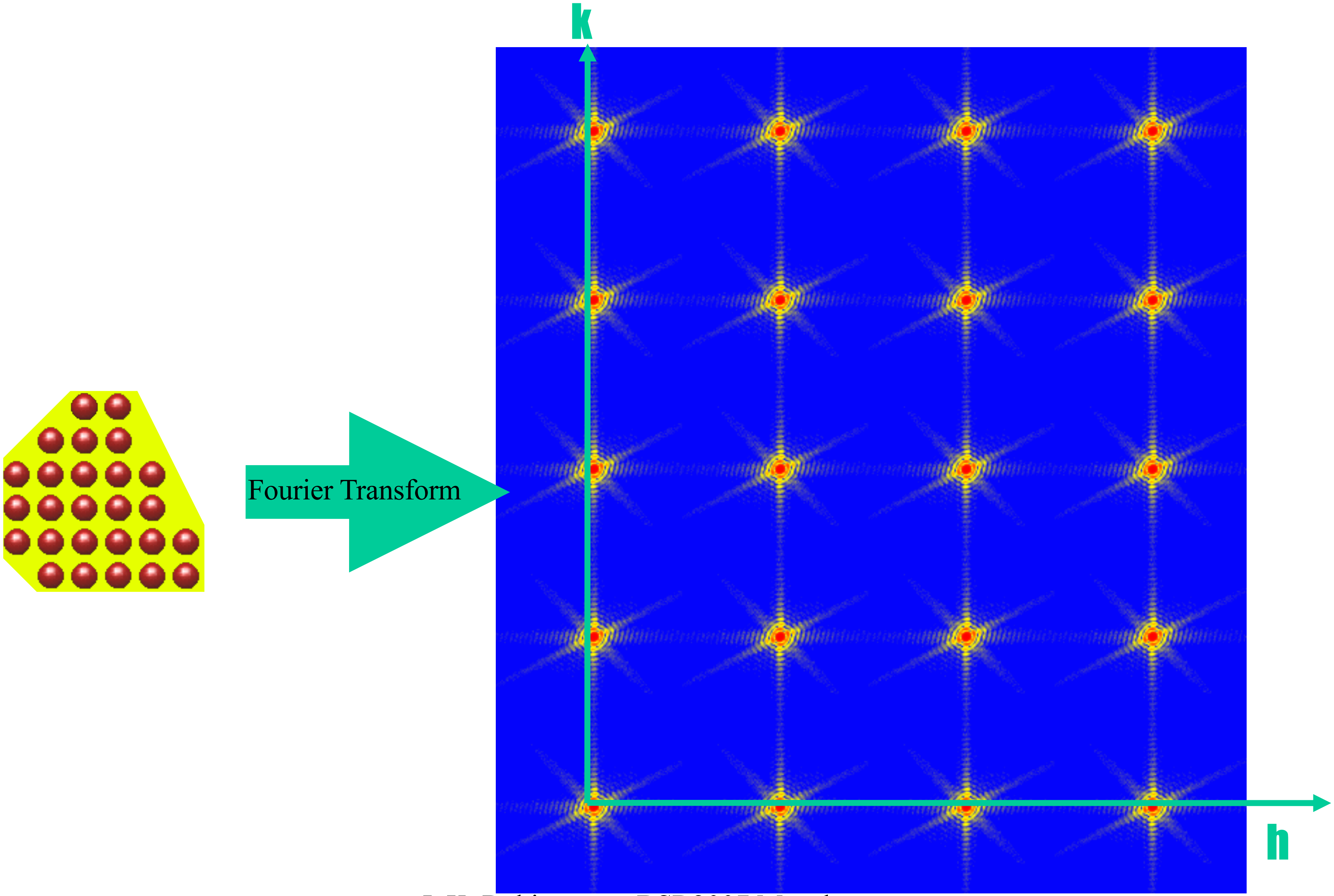


Lensless X-ray Microscope





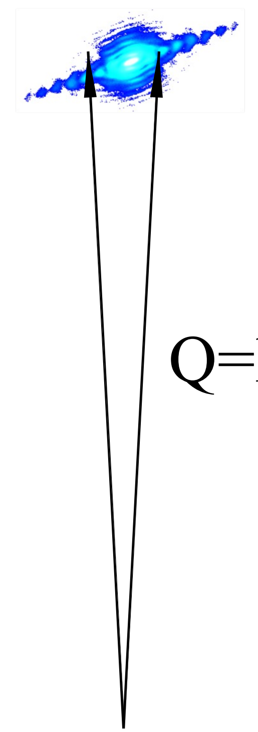
Coherent Diffraction from Crystals



3D Diffraction Method

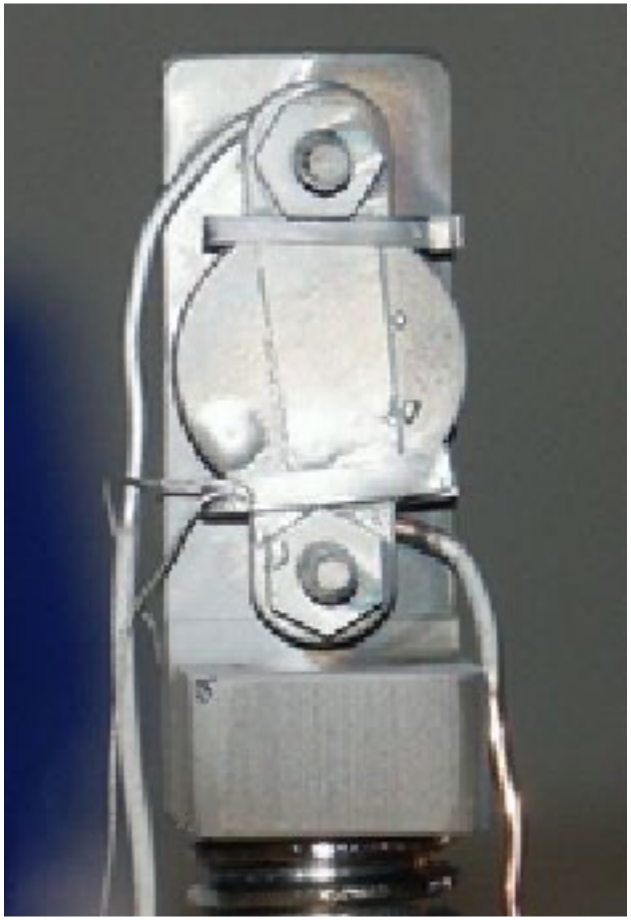
k_f

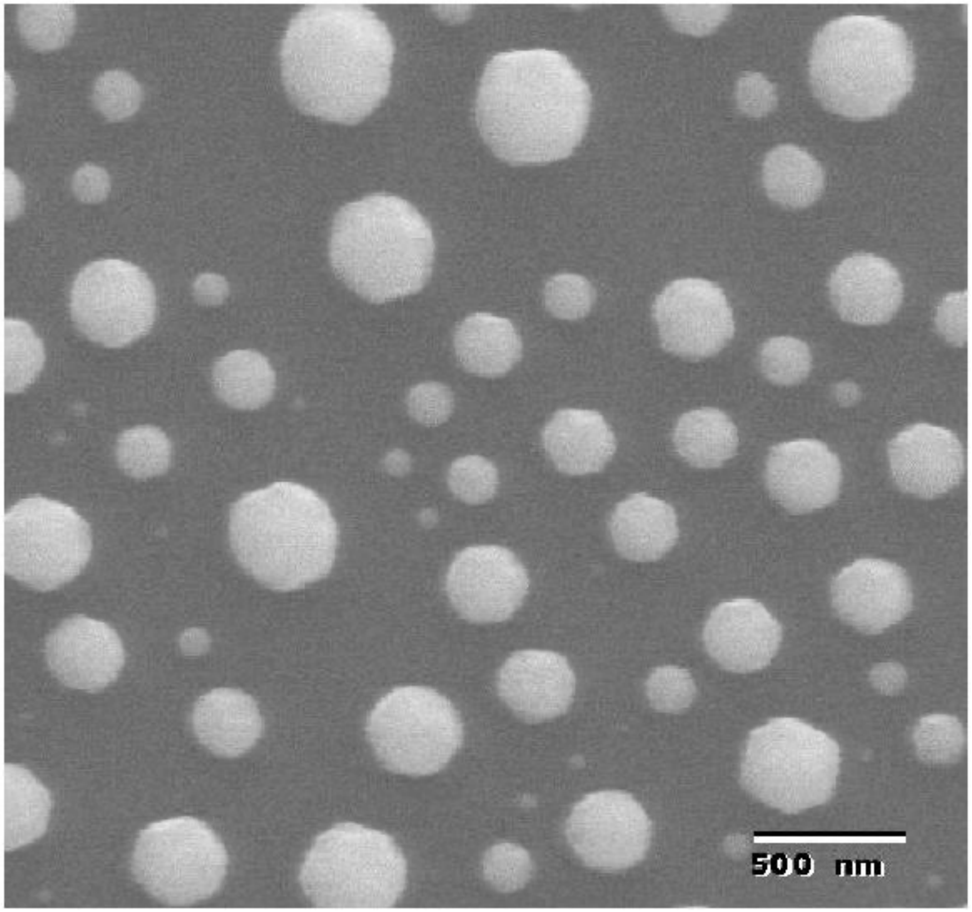
k_i



$Q=k_f - k_i$

In situ growth of Pb crystals





Good statistics, 3D diffraction data

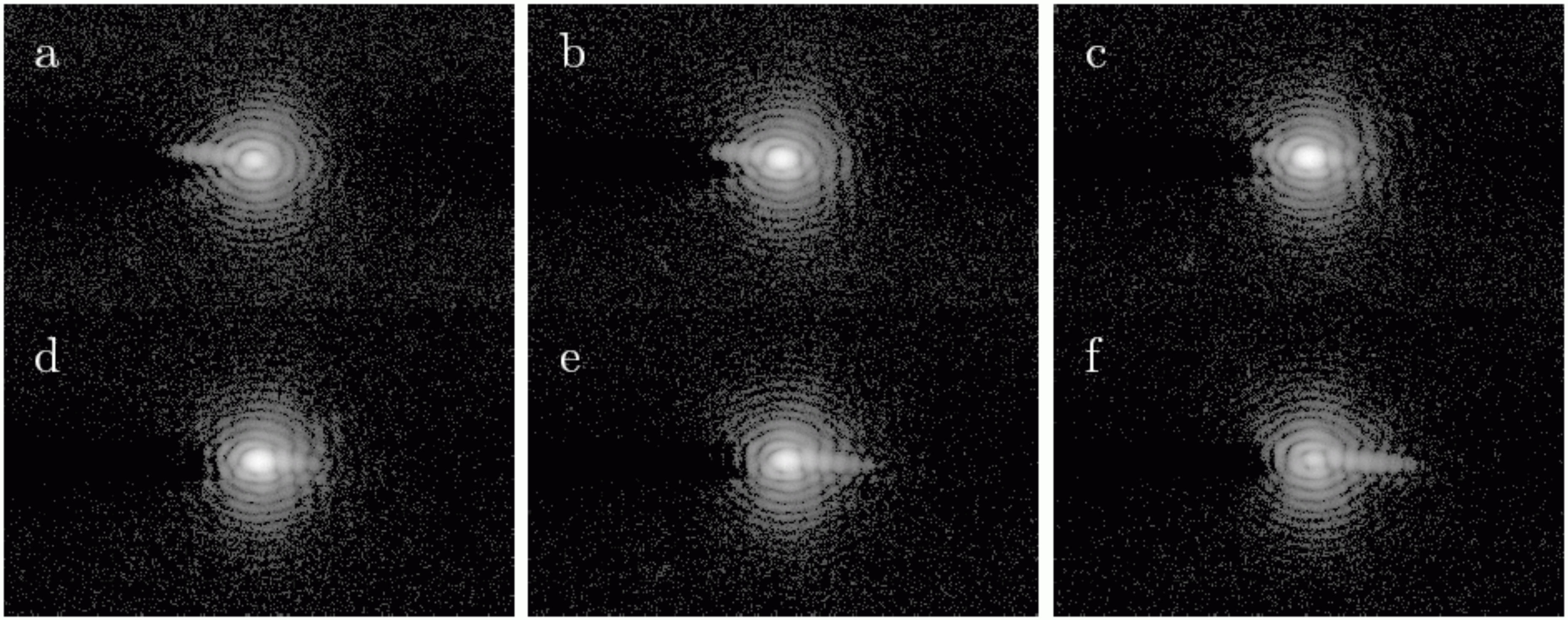
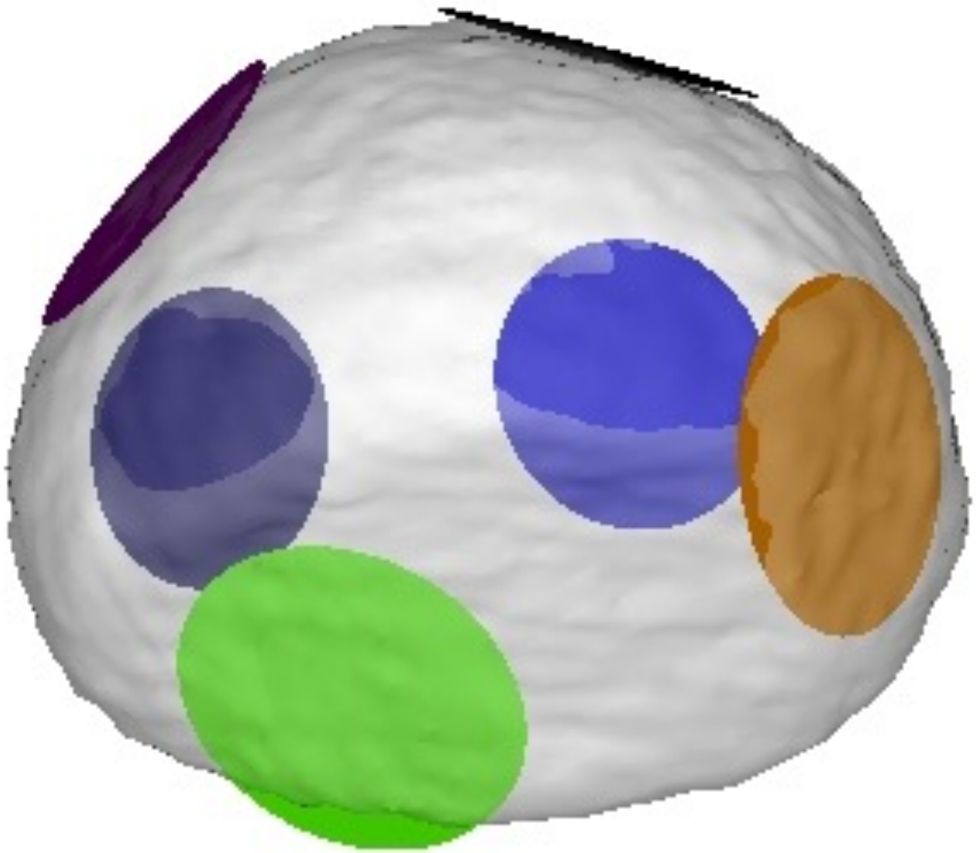
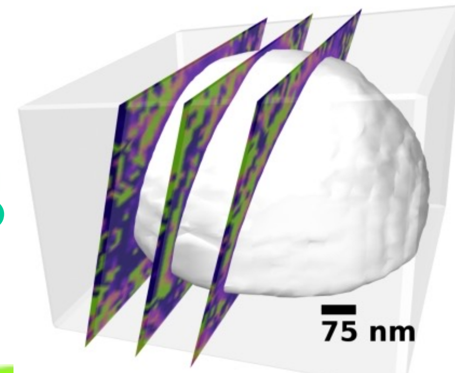


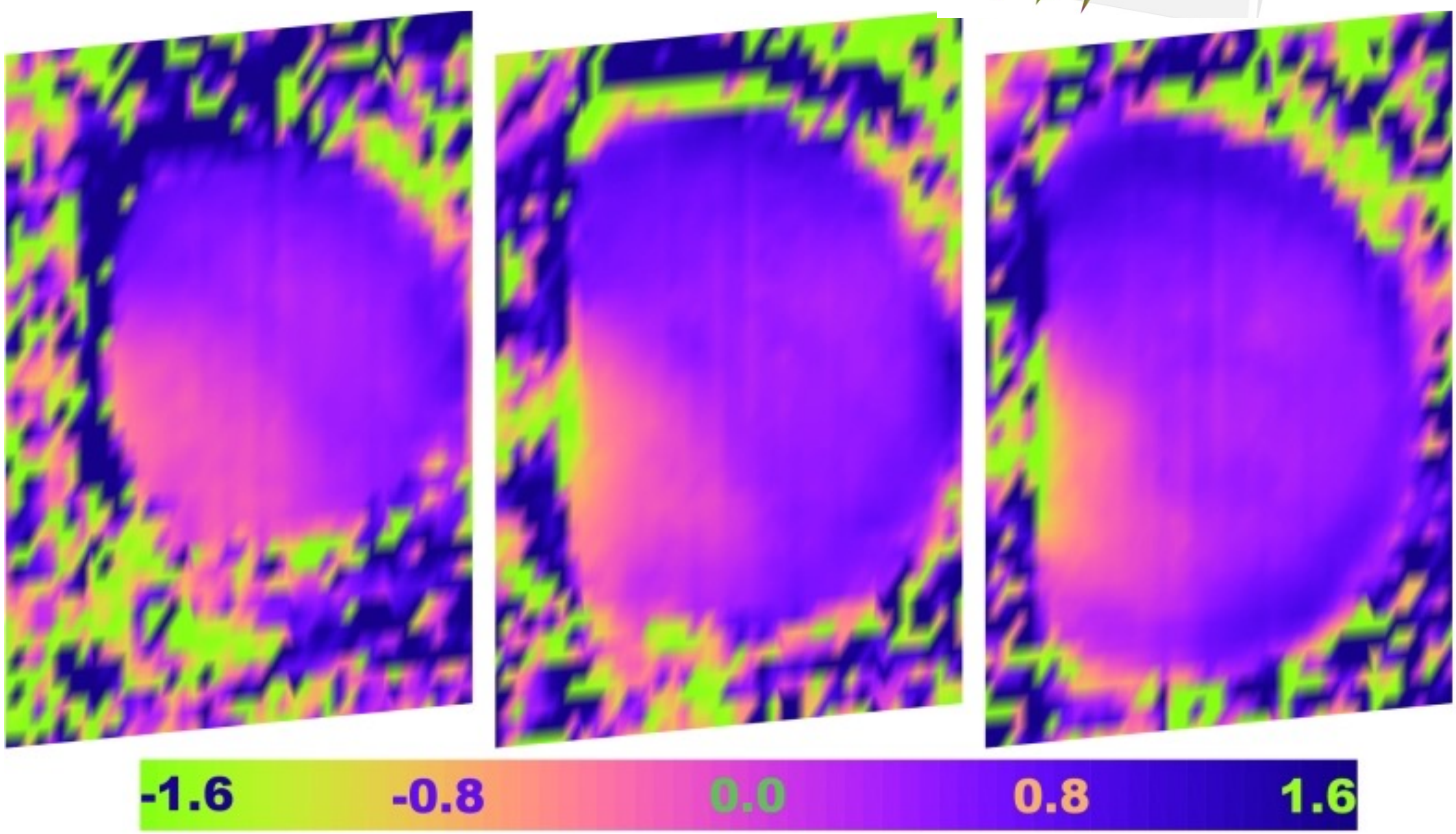
Figure 4.12: Center slices from 3D CXD pattern from Pb sample, on a log scale. Data file 296 from 10/03.

Facets of Equilibrium Crystal Shape

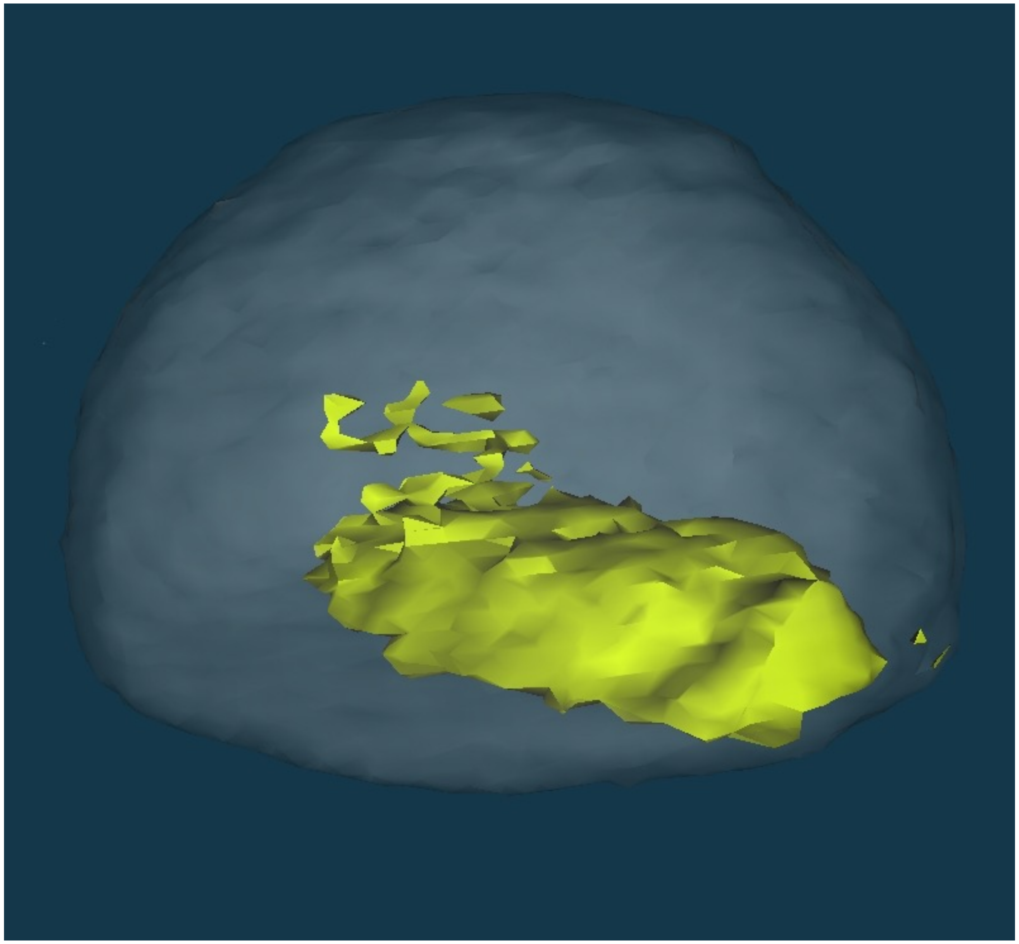


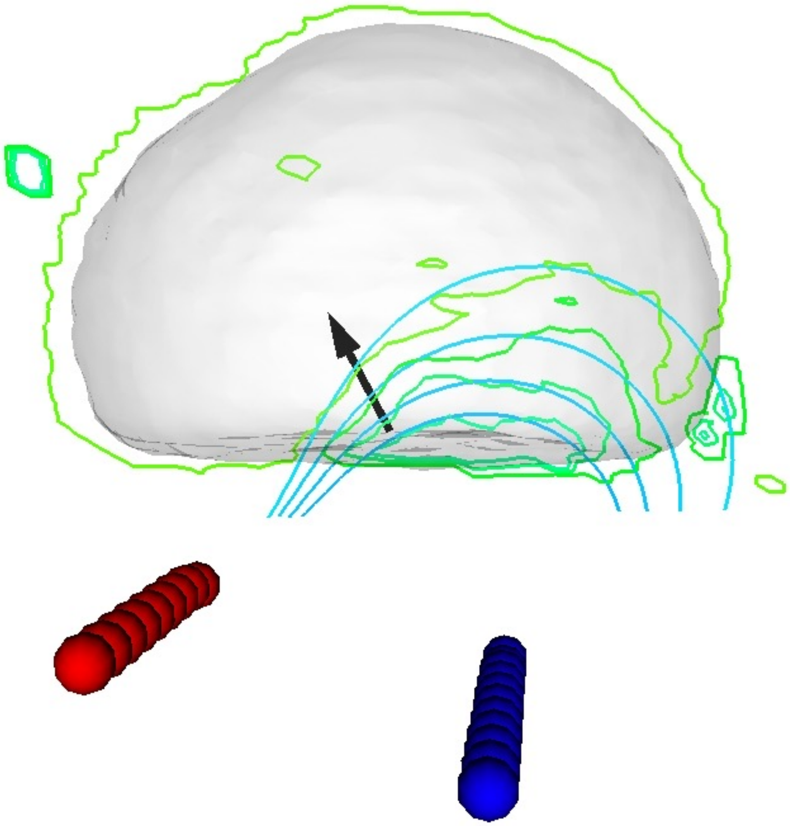
3D phase map sections

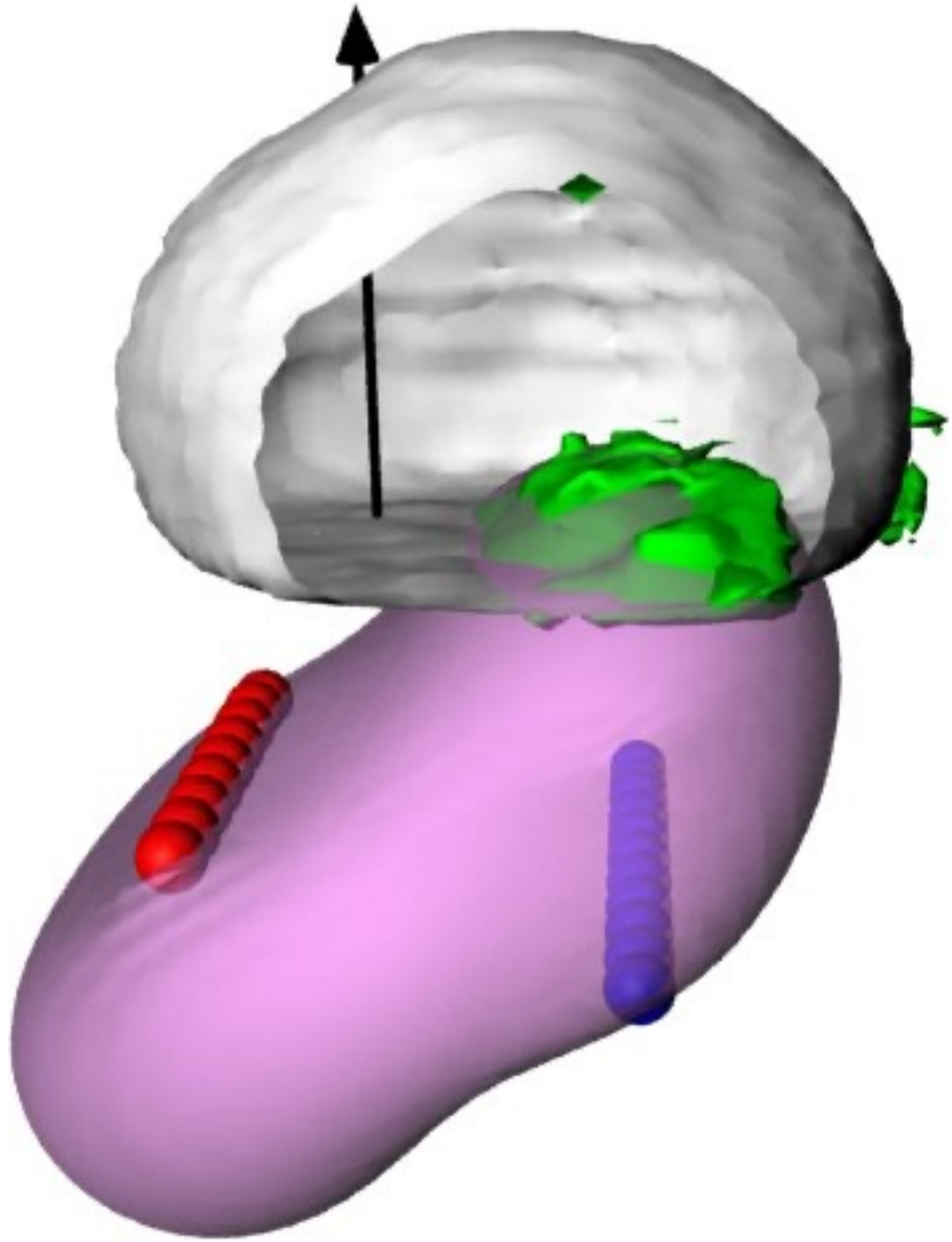




Modeling of 3D Phase Bump







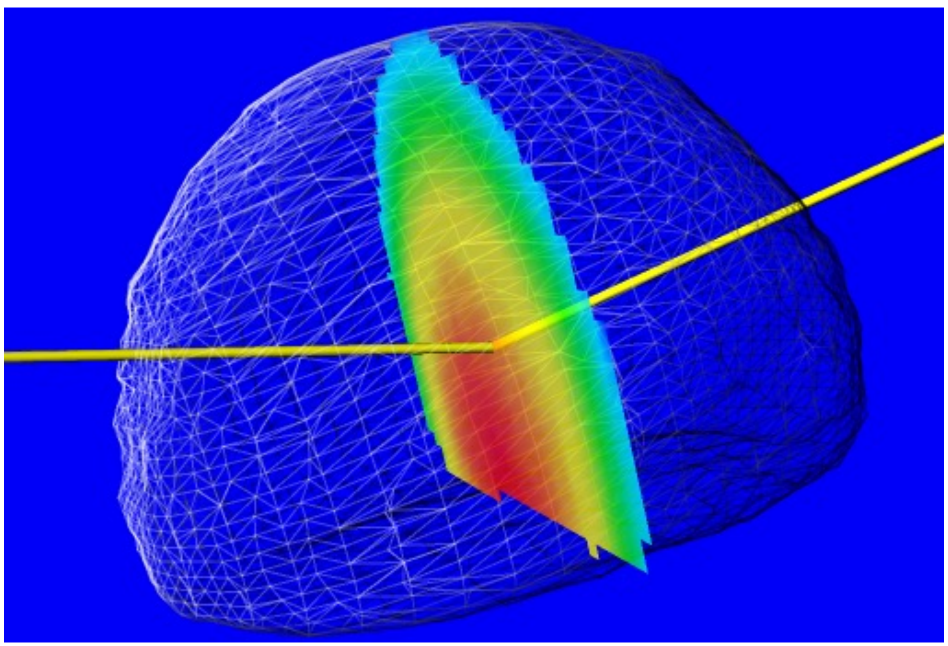
Refraction effects in Lead at 8.9keV

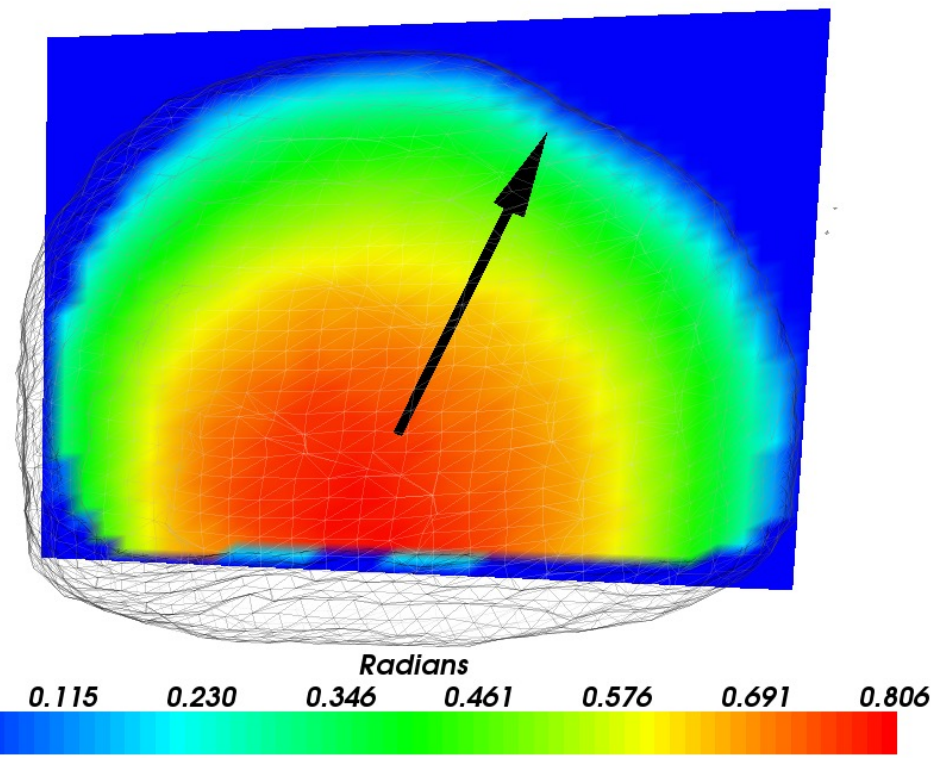
Phase accumulation due to refraction along scattering path

d=750nm: $kd\delta = 0.76$rad

$kd\beta = 0.07$

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





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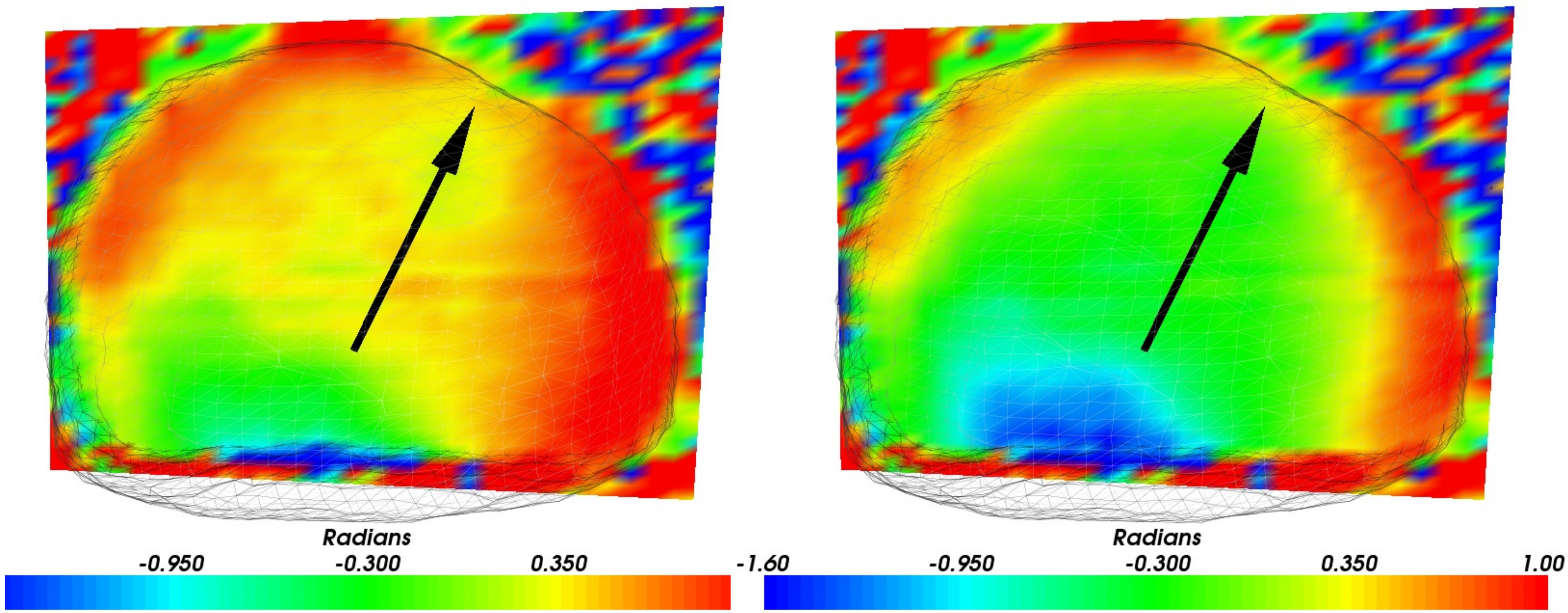
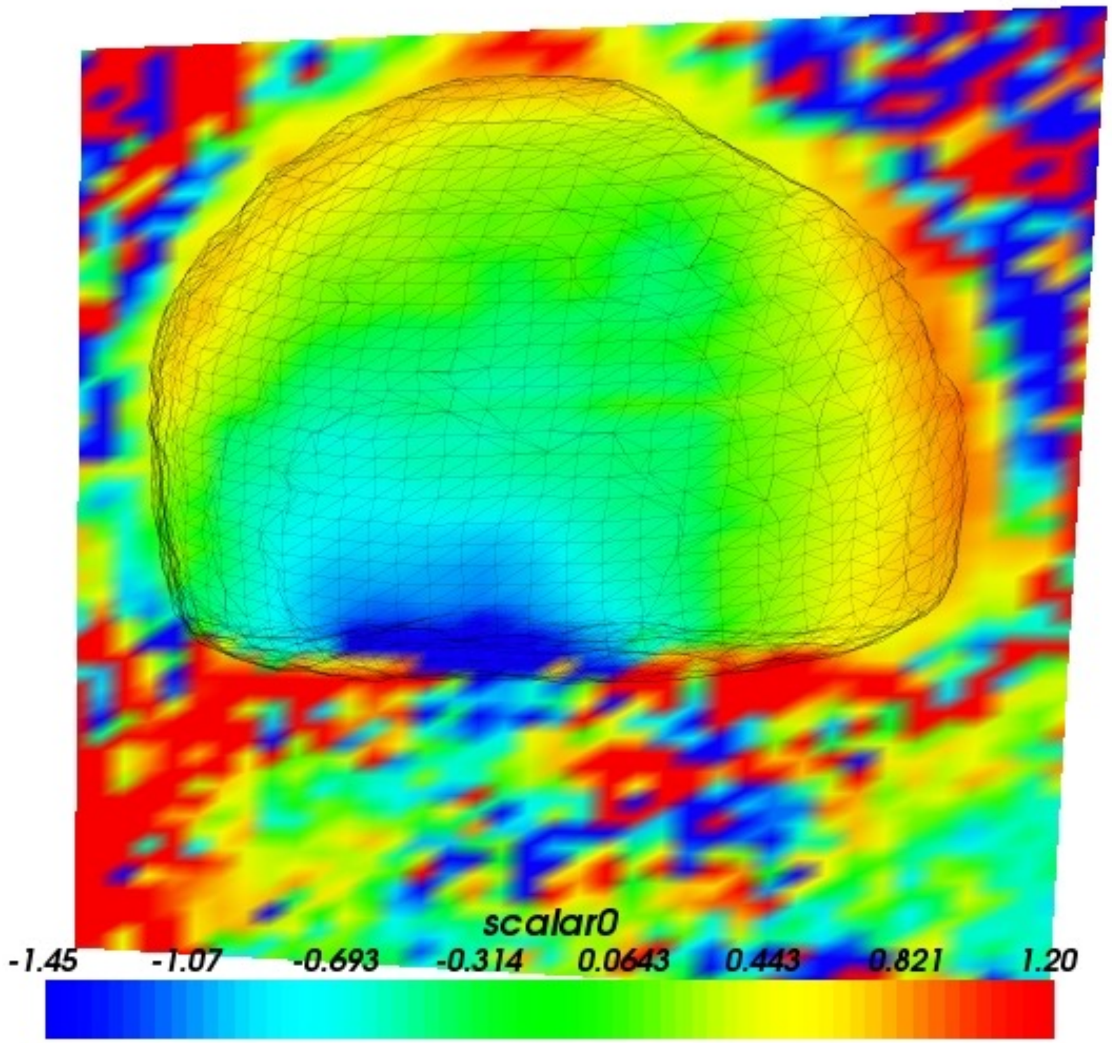
Phase Maps before and after Correction



Positive Phase is Surface Expansion

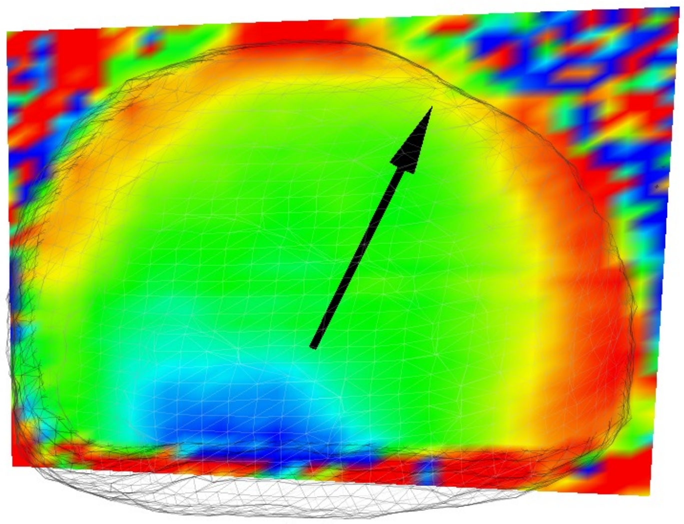
including correction for refraction by crystal

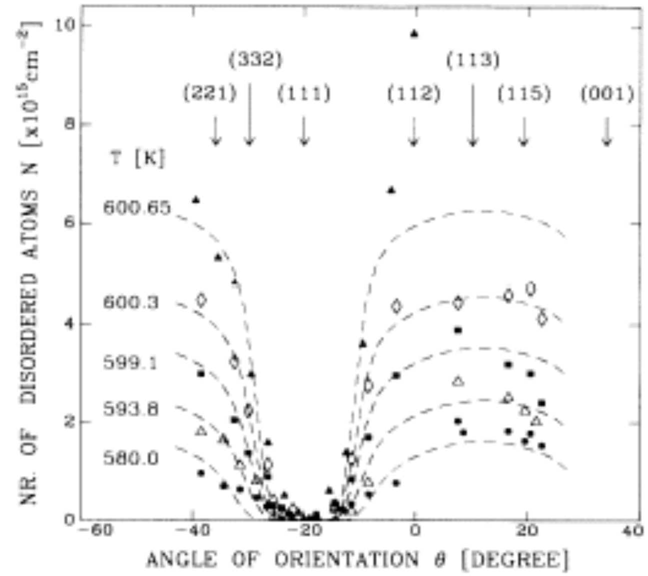


Max phase = 1.15rad
= 0.052nm

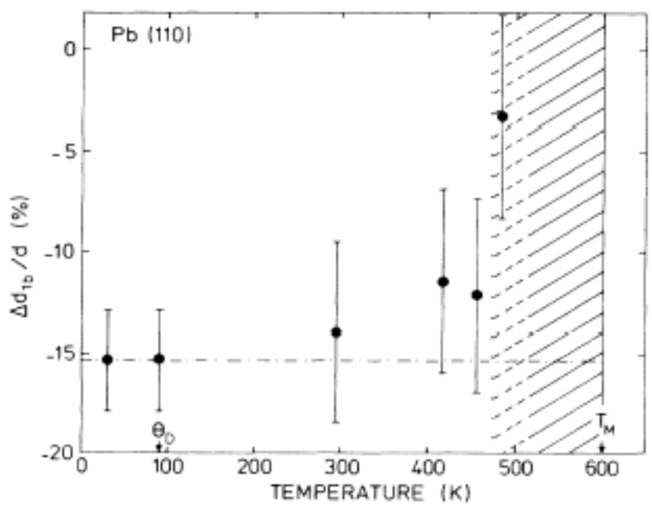
Phase on (111) facet:
= 0.47 rad
= 0.02nm

Surface Thermal Expansion: ion channeling + theory

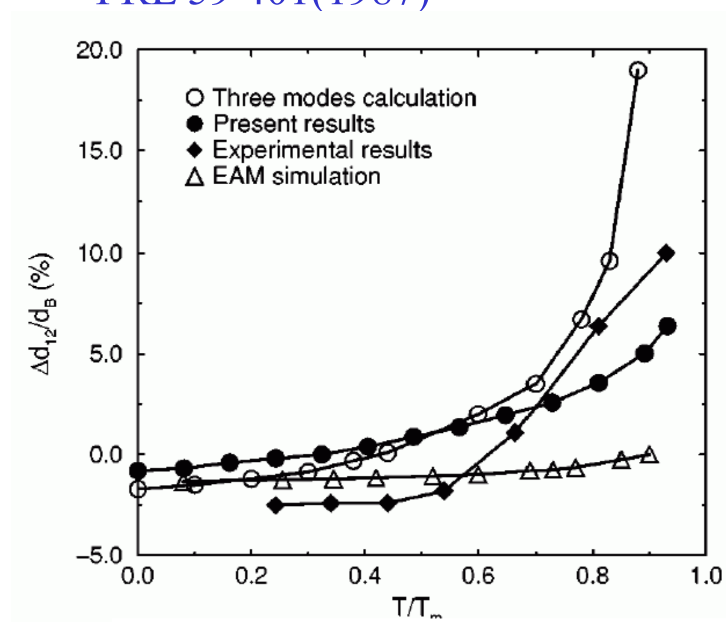




Pluis, van der Gon, Frenken, van der Veen
PRL 59 401(1987)



Frenken, Huusen, van der Veen
PRL 58 (1987)

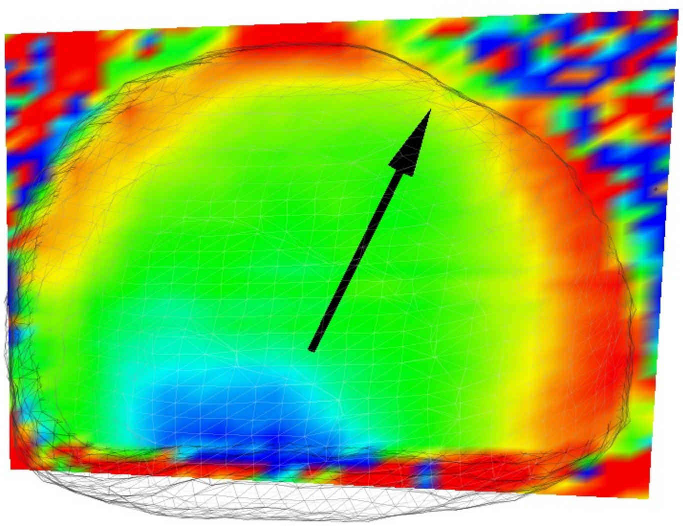


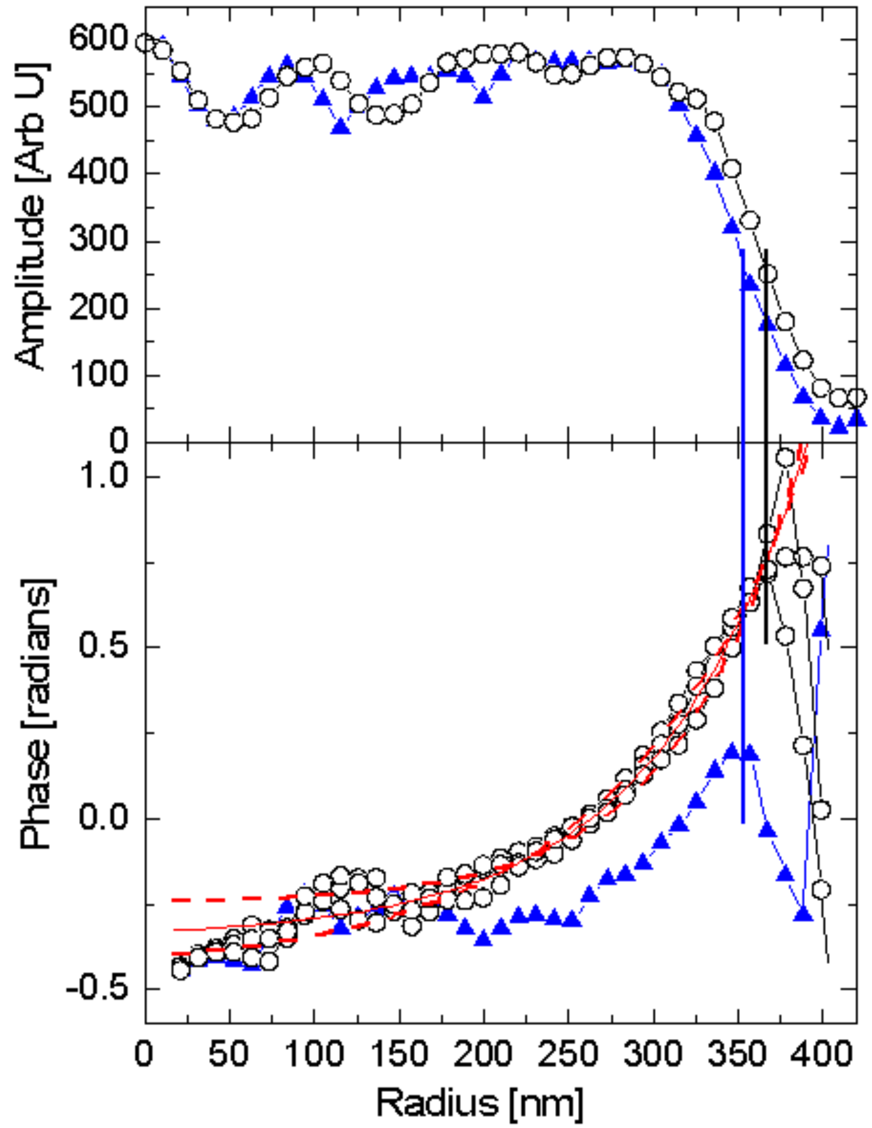
Ag(111) theory J. Xie (Scheffler group)
PRB 59 970 (1999)

Depth Variation of Distortion

Facets subtend 18.6 ±2.6°
ECS predicts 14°

Depth measured 90 ± 20nm
Theory says 75nm





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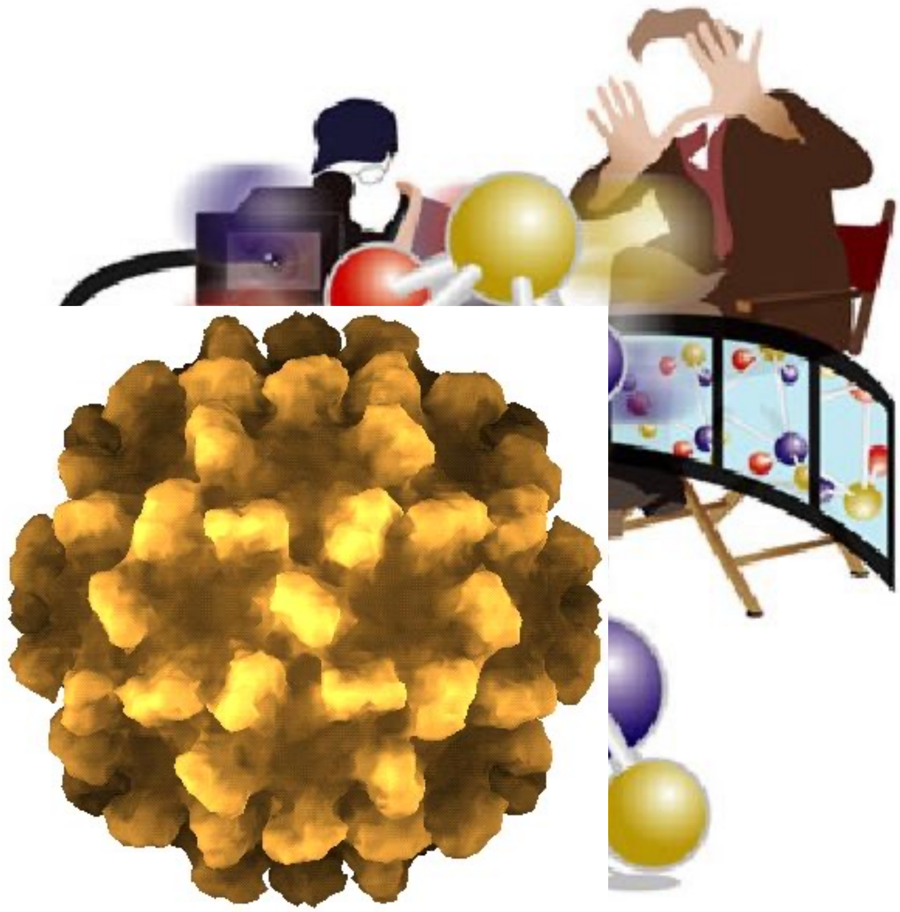
Molecular Movies using XFEL



I. K. Robinson BSR2007 Manchester

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!

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