Solution Scattering Studies of Protein Crystallization

- Ian Robinson
- Sébastien Boutet
- Jing Tao
- Jim Zuo

Departments of Physics and Materials Science
University of Illinois

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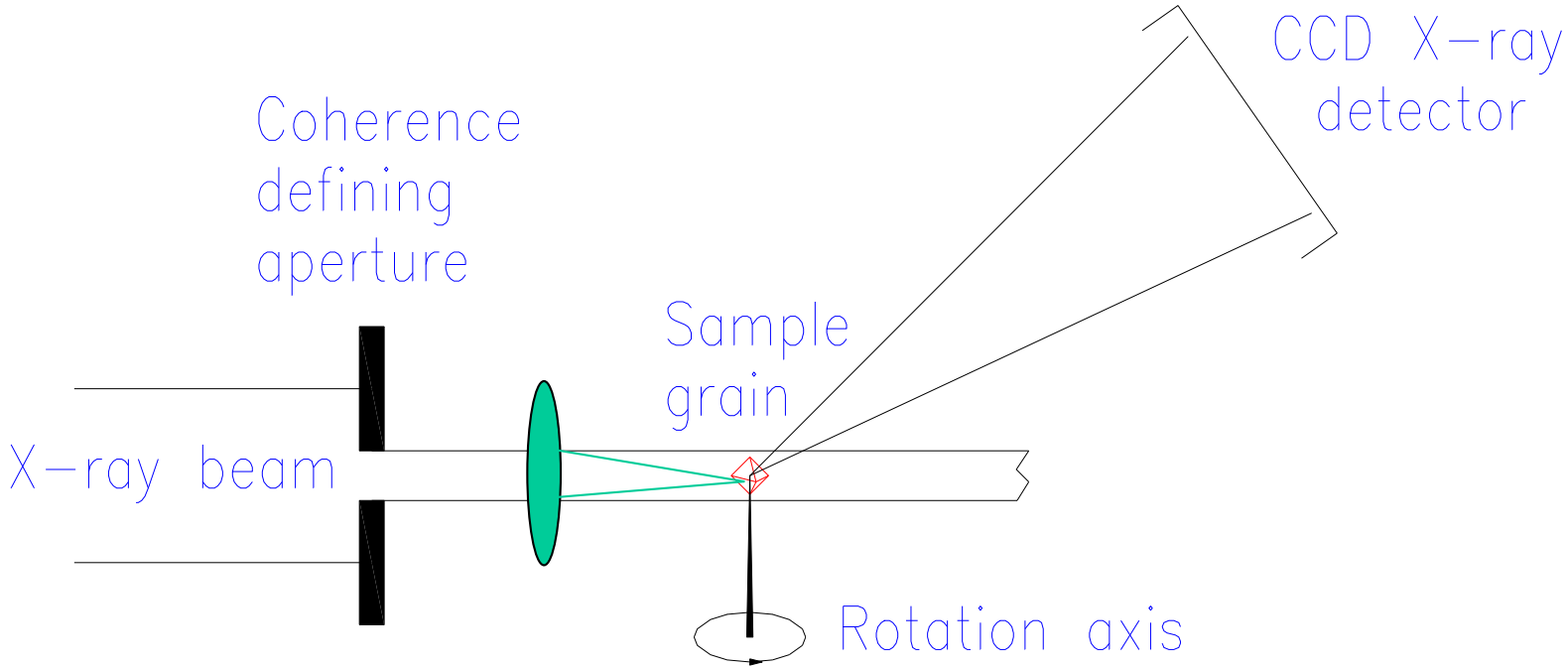
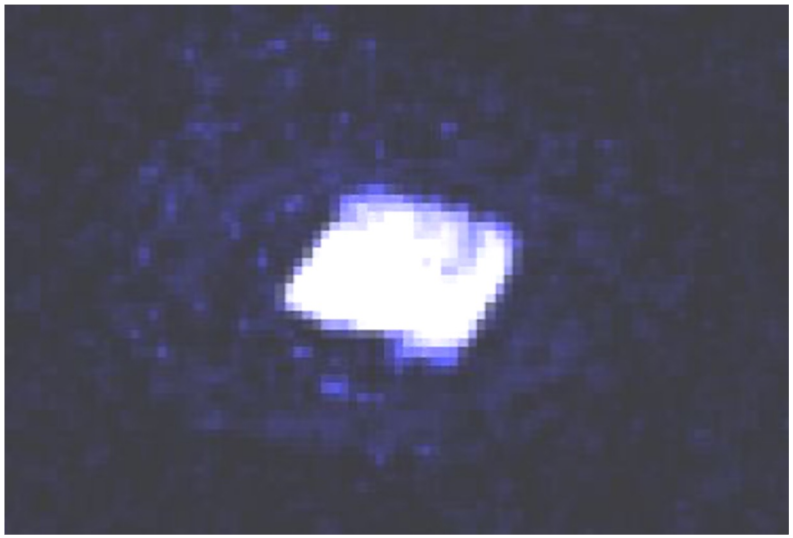
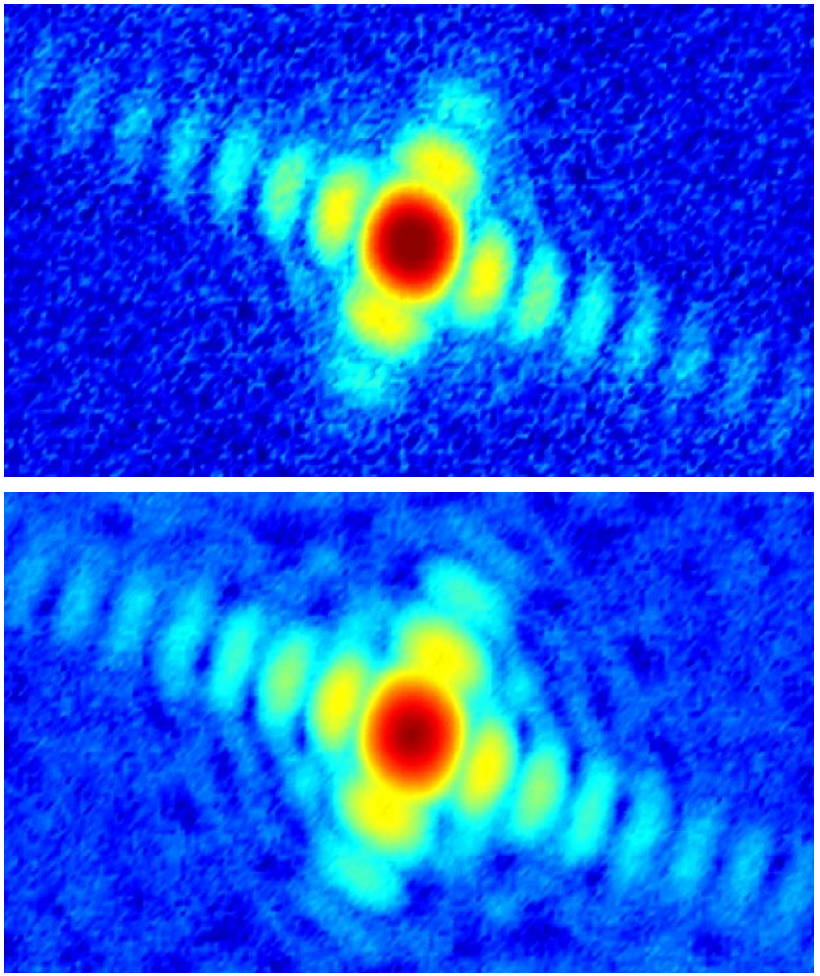
Lensless X-ray Microscope



Reconstruction of Ag Nanocrystal

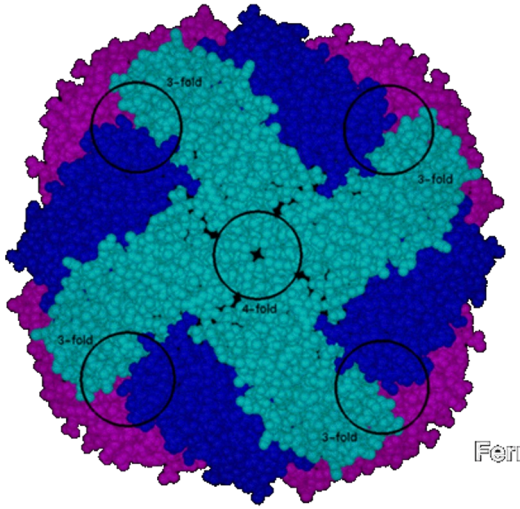


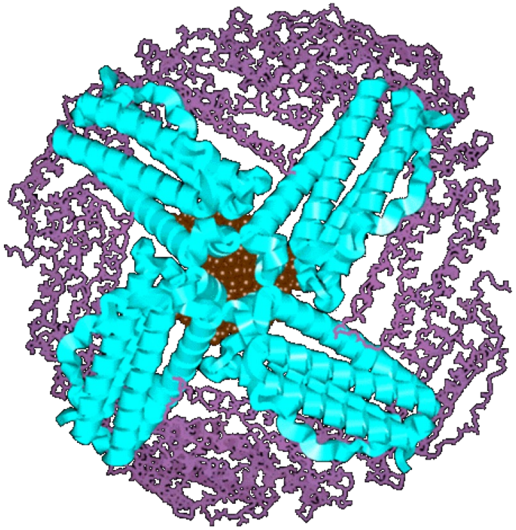
200nm



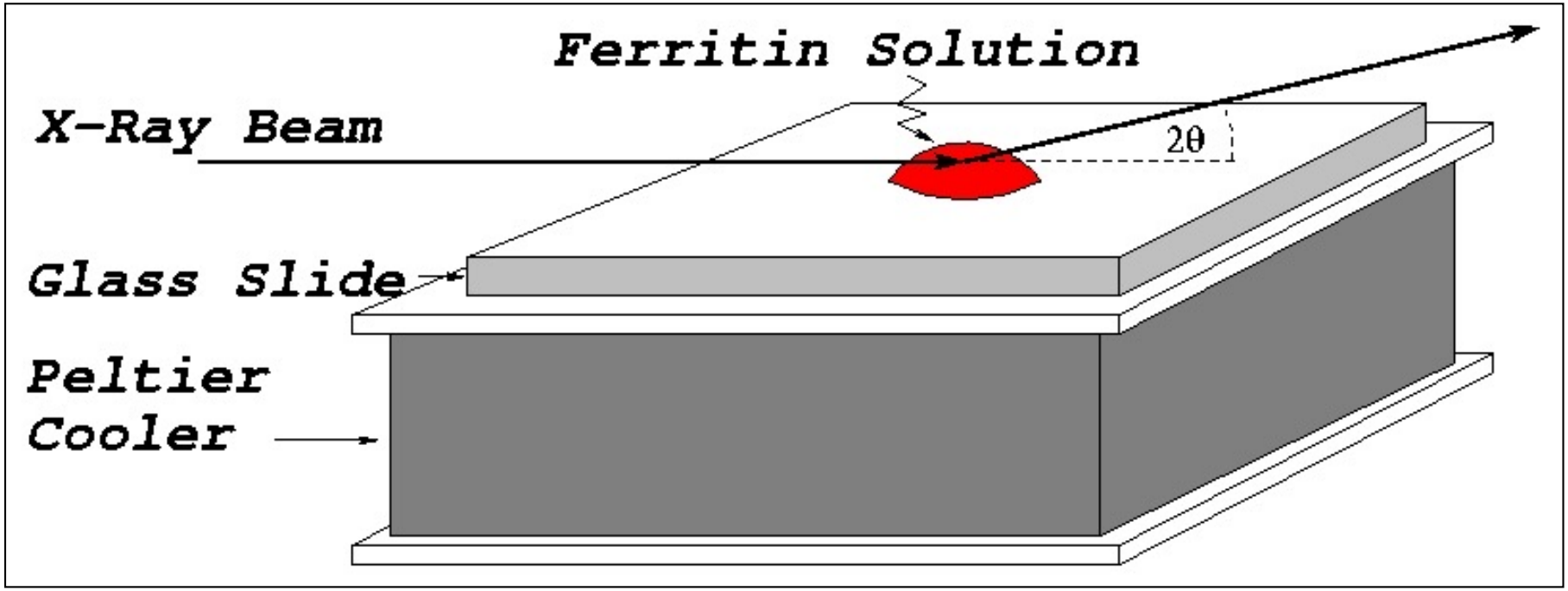
Horse Spleen Ferritin

- 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

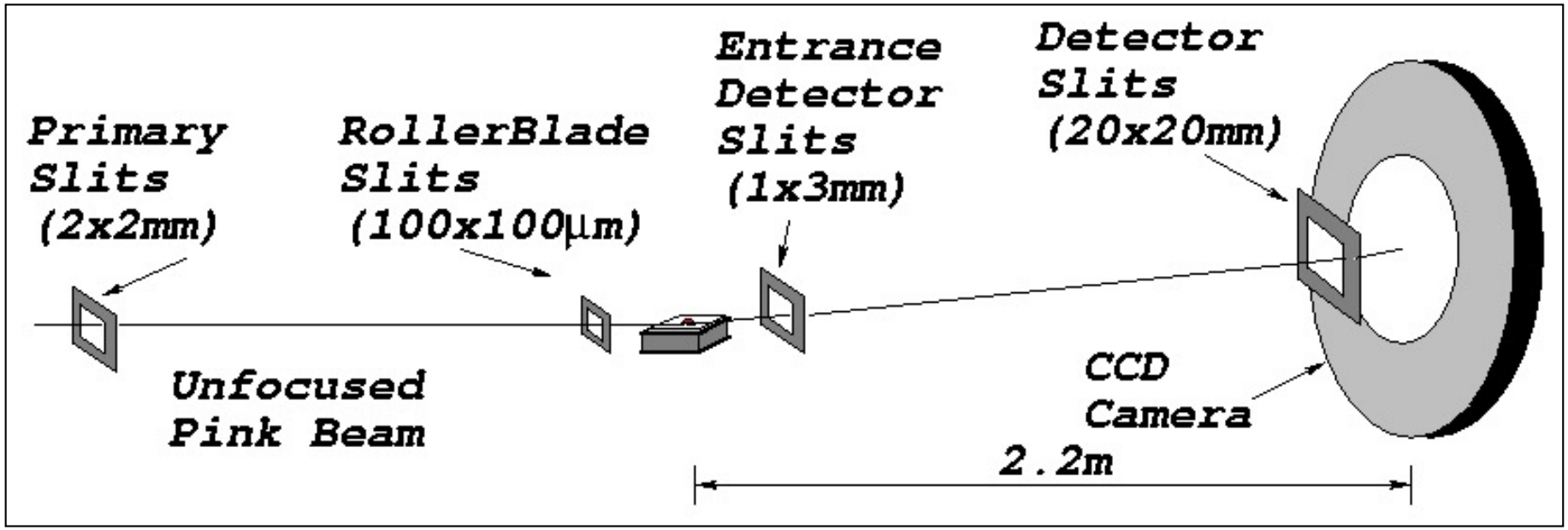




In-situ Study of Crystallization

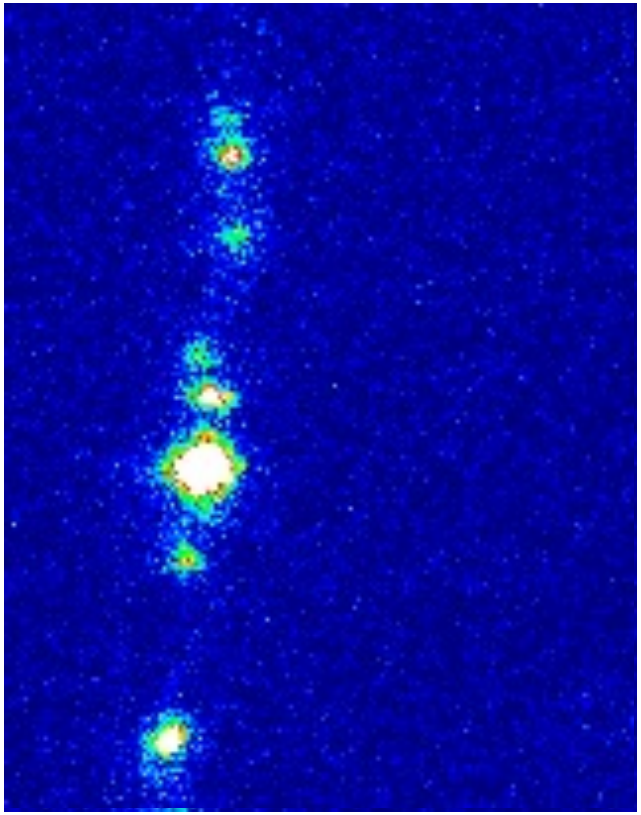


Experiment at APS Sector 34



Ferritin (111) Powder Ring

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



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.

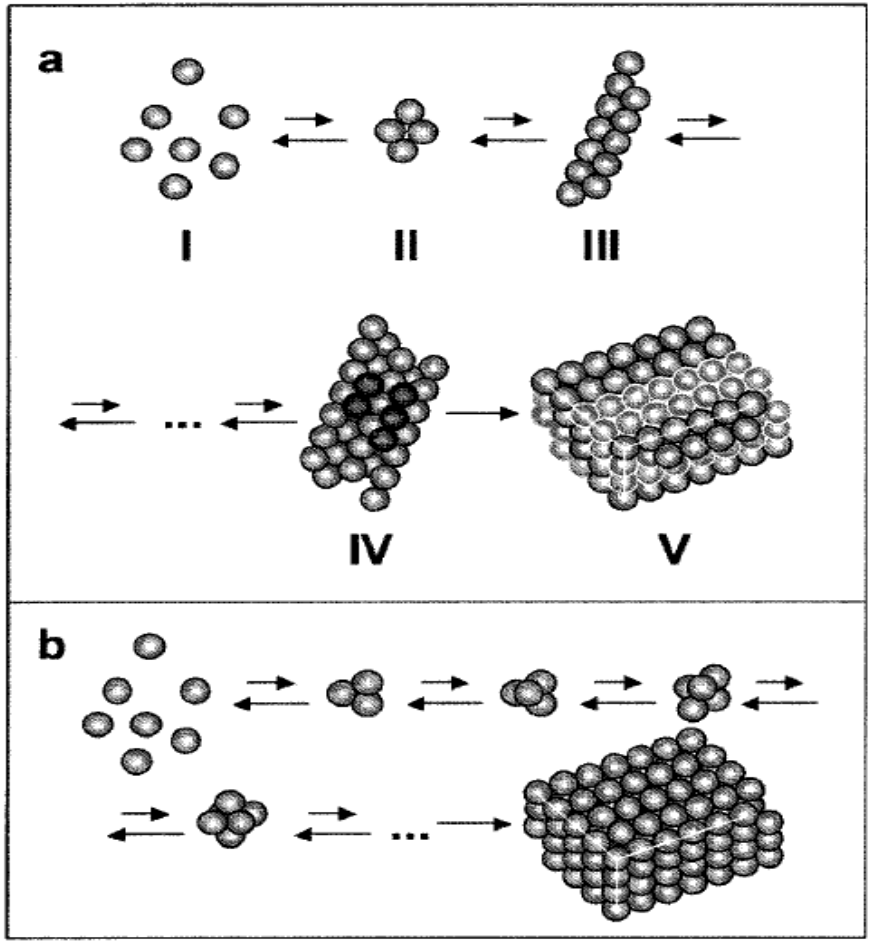
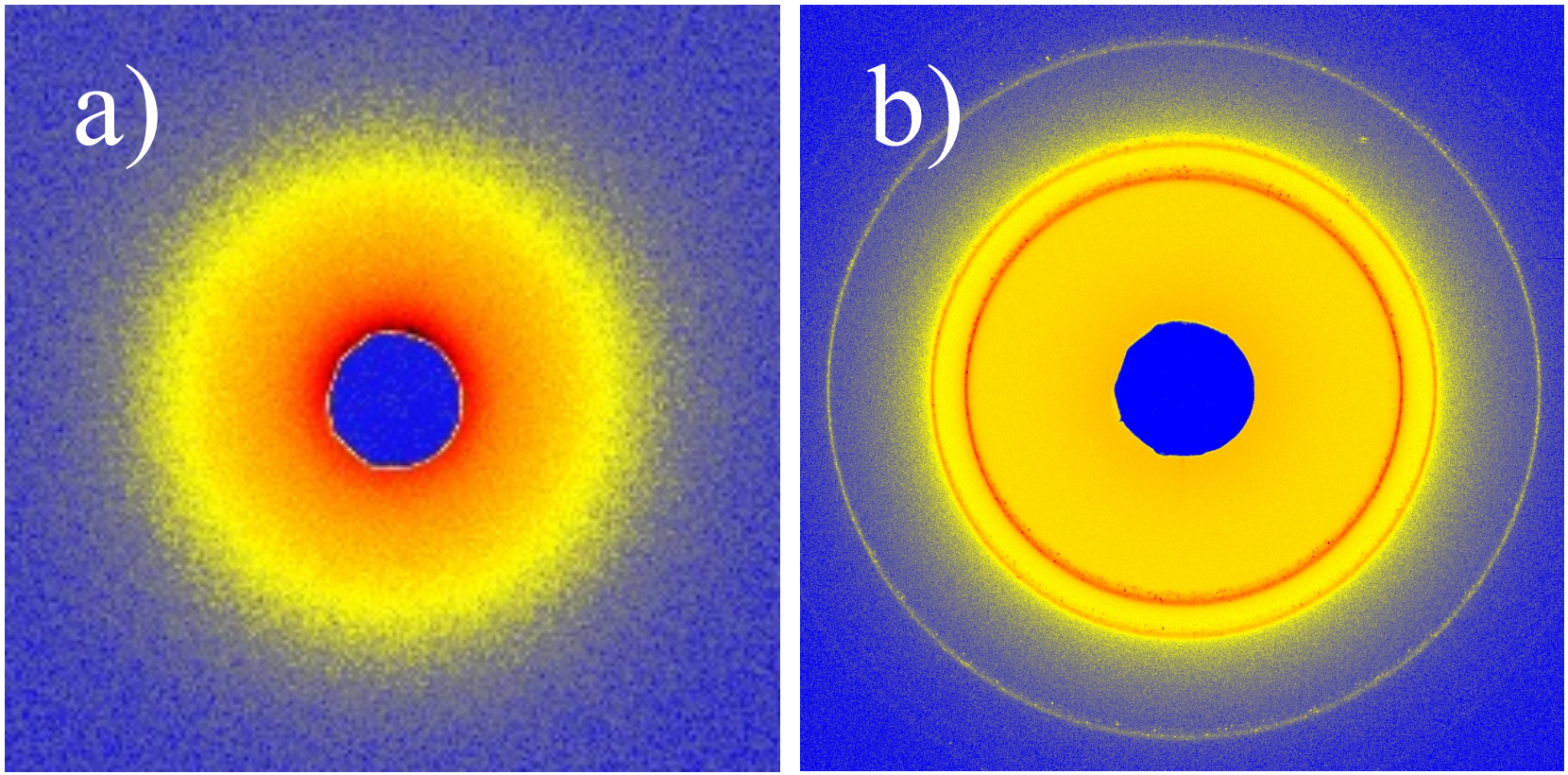


Figure 10. Schematic illustration of two nucleation pathways: (a) via a planar critical cluster (in IV molecules belonging to the second layer are shown in a lighter shade; in V the (110) layers that stack up to form this crystal are delineated by lighter and darker contours) and (b) via compact critical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

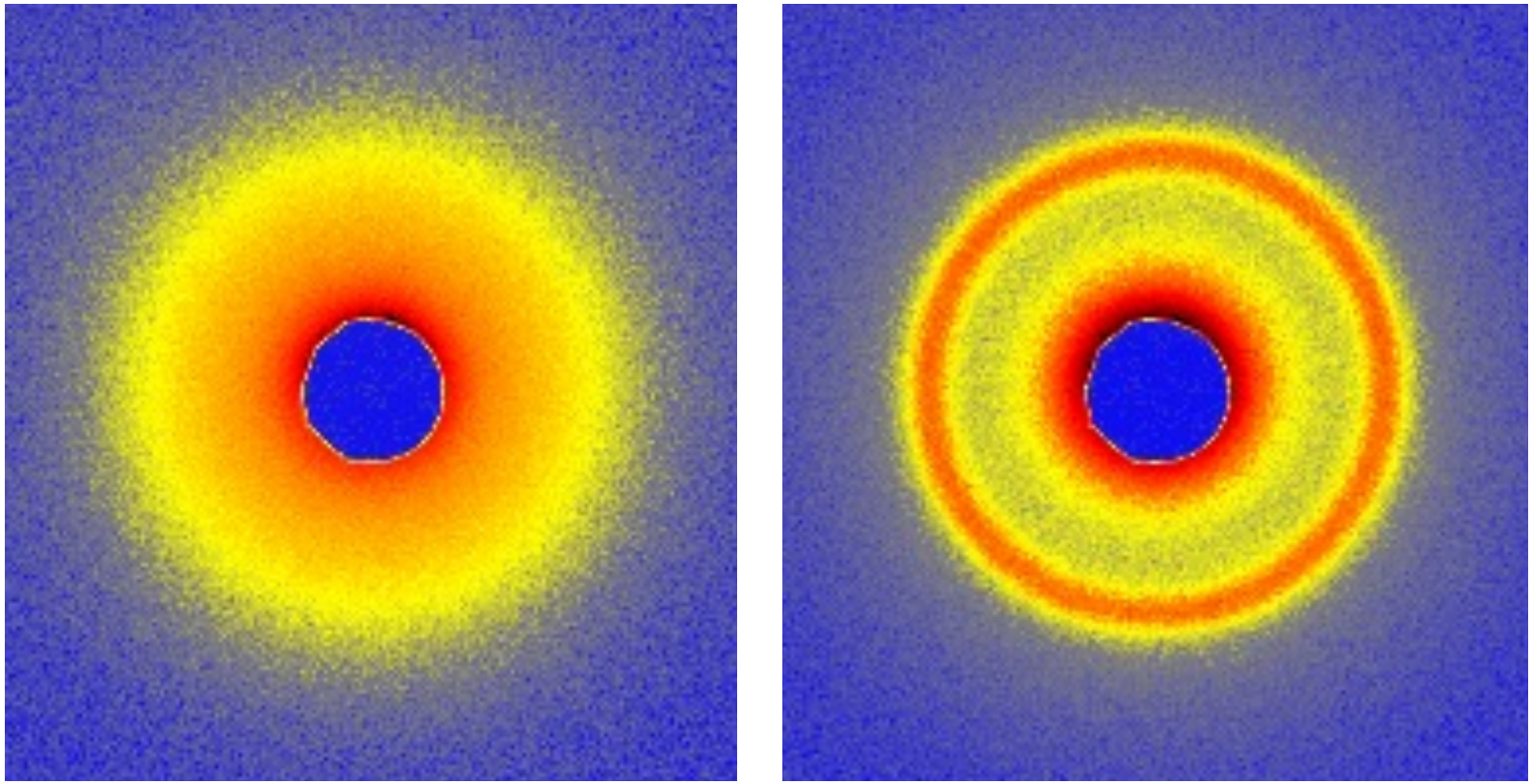
Crystallization of Holoferritin: SAXS



a) SAXS pattern of holoferritin at 10°C.

b) SAXS pattern after adding Cadmium salt

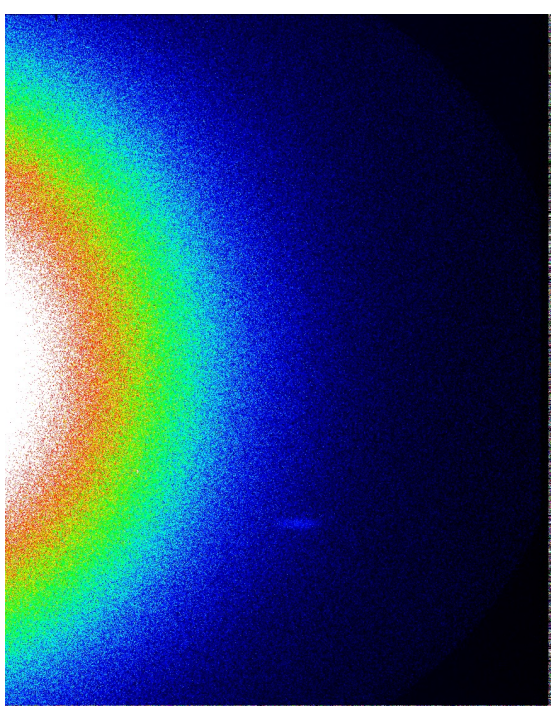
Effect of Temperature



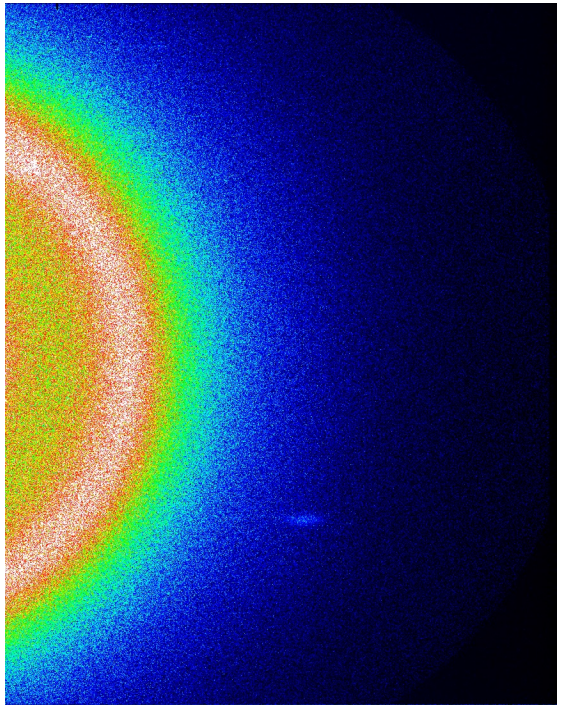
SAXS pattern at T=+10°C and -10°C.

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

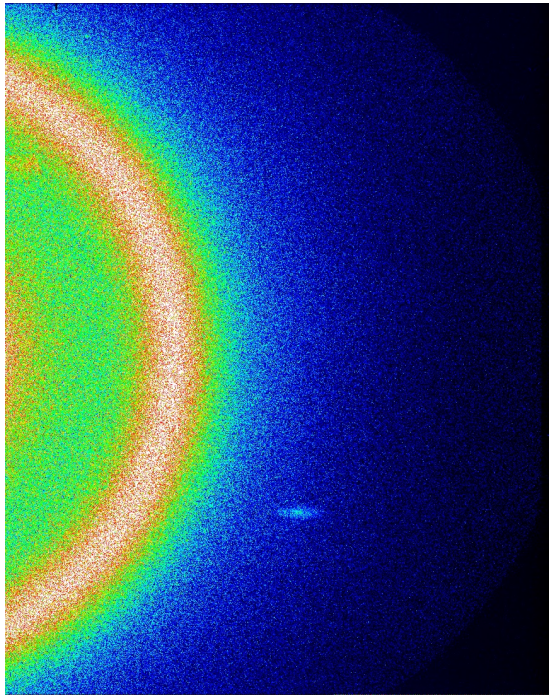
Ferritin Solution upon Freezing



T=13.3

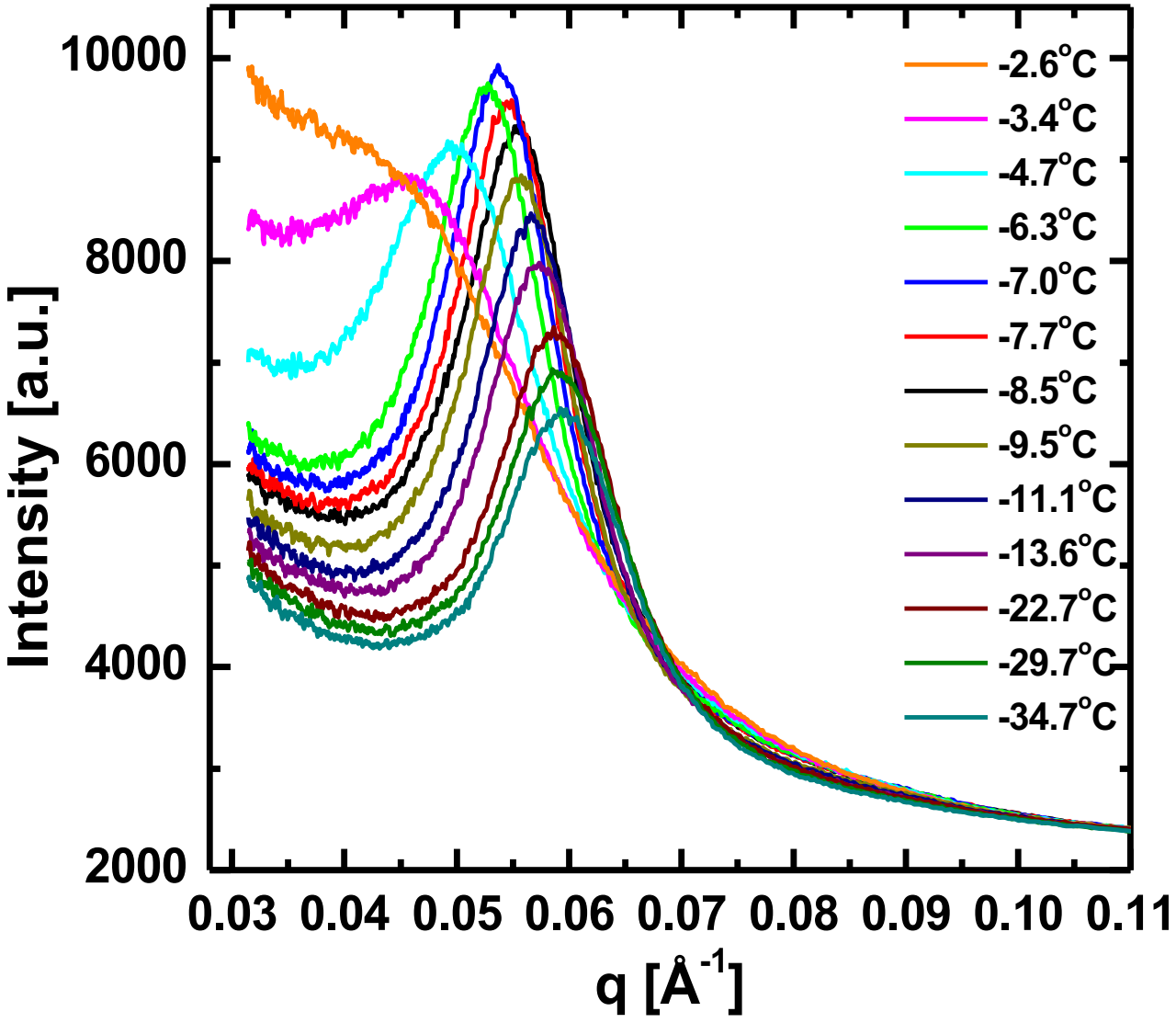


T=-4.7



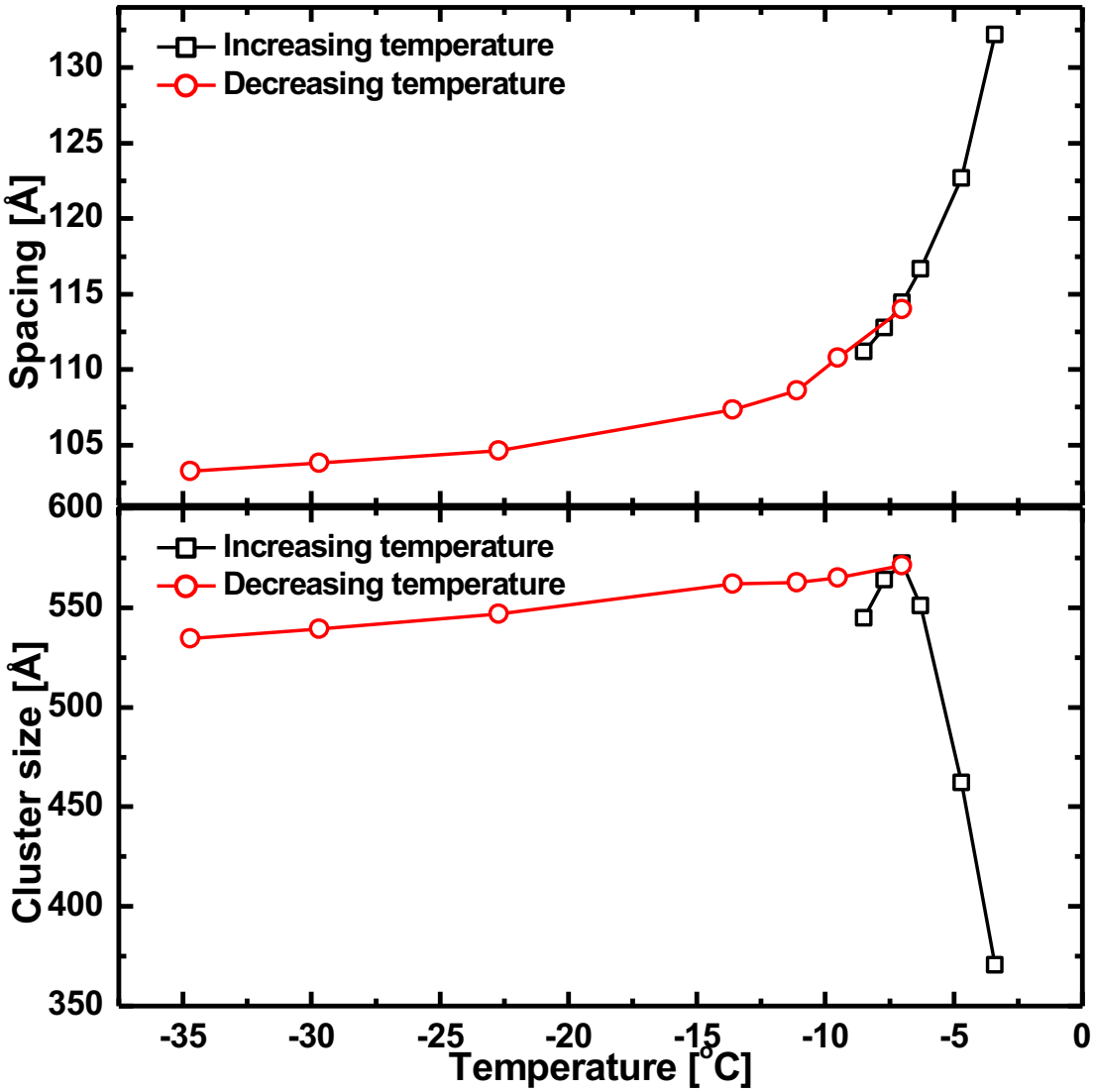
T=-34.7

Integrated SAXS Data



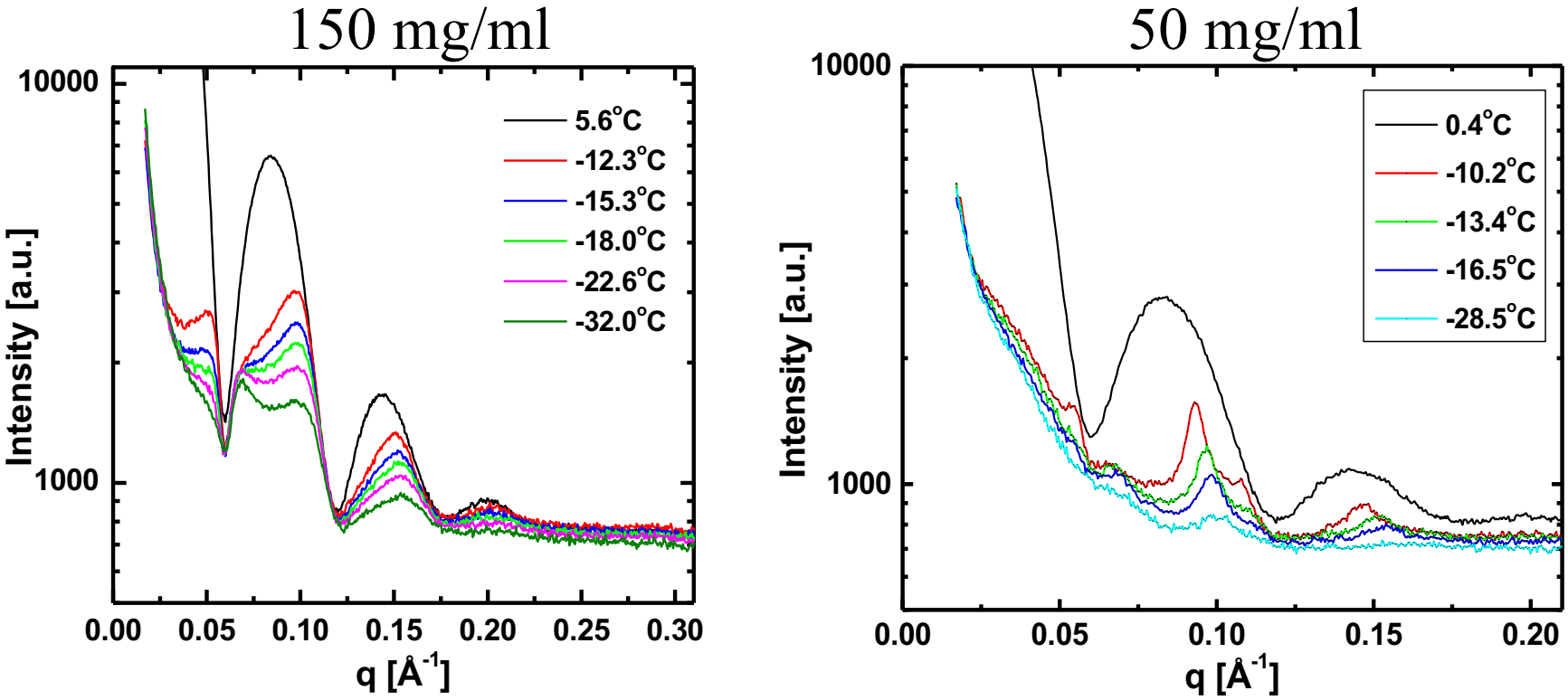
- The SAXS pattern shows a broad peak which shifts in position and changes in width as a function of temperature

Thermal "Expansion"



- The position and width of the peaks were fitted with Gaussians.
- The solution freezes and melts at different temperatures.
- The spacing of the molecules in the clusters varies by 30% over 30°C

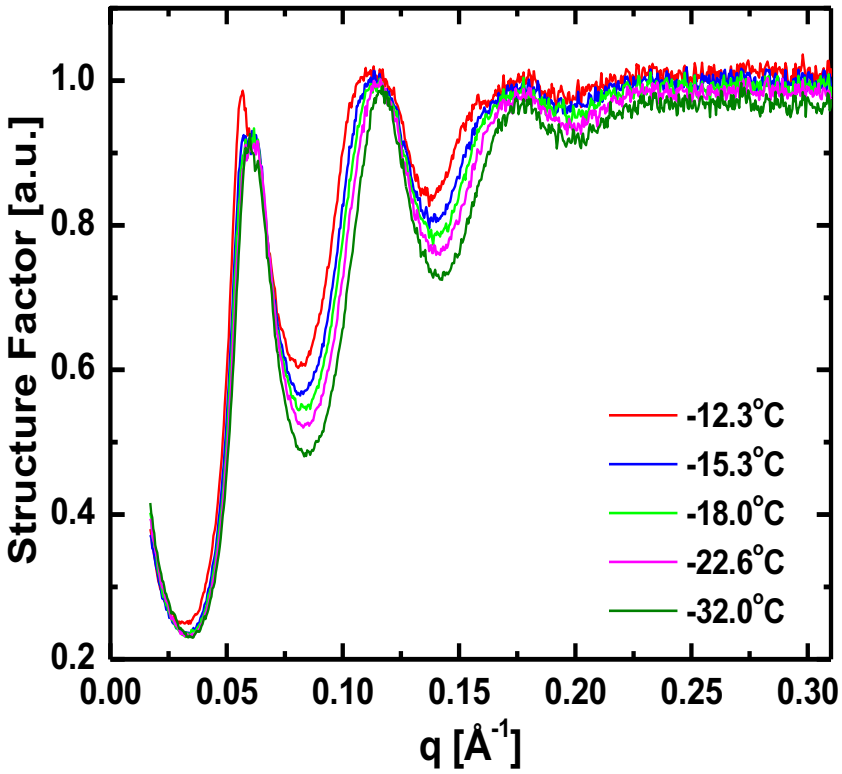
Apoferritin Data



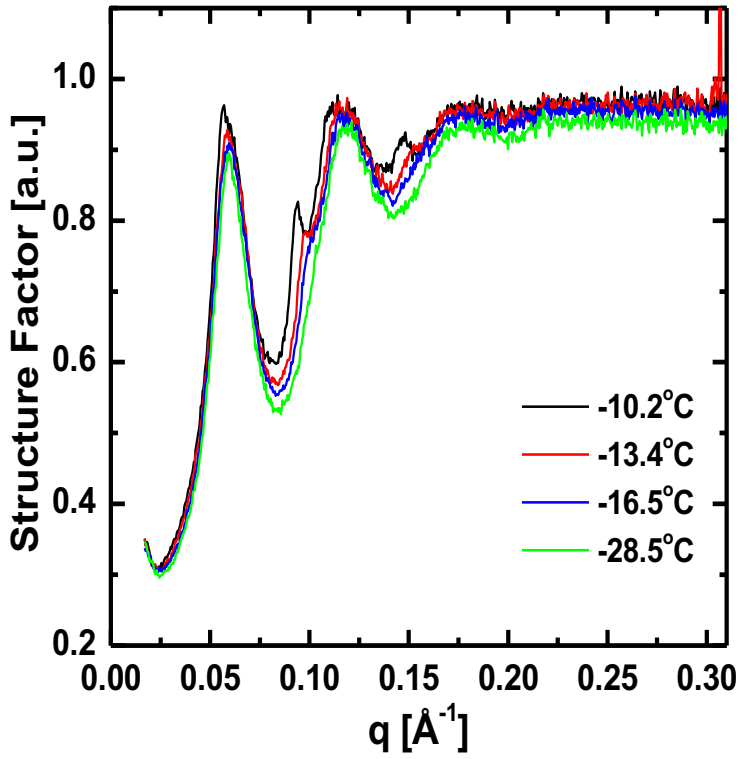
- The SAXS pattern of apoferritin changes drastically when frozen, from the Bessel function like curve to sharper, but weaker peaks.

Apoferritin Structure Factors

150 mg/ml



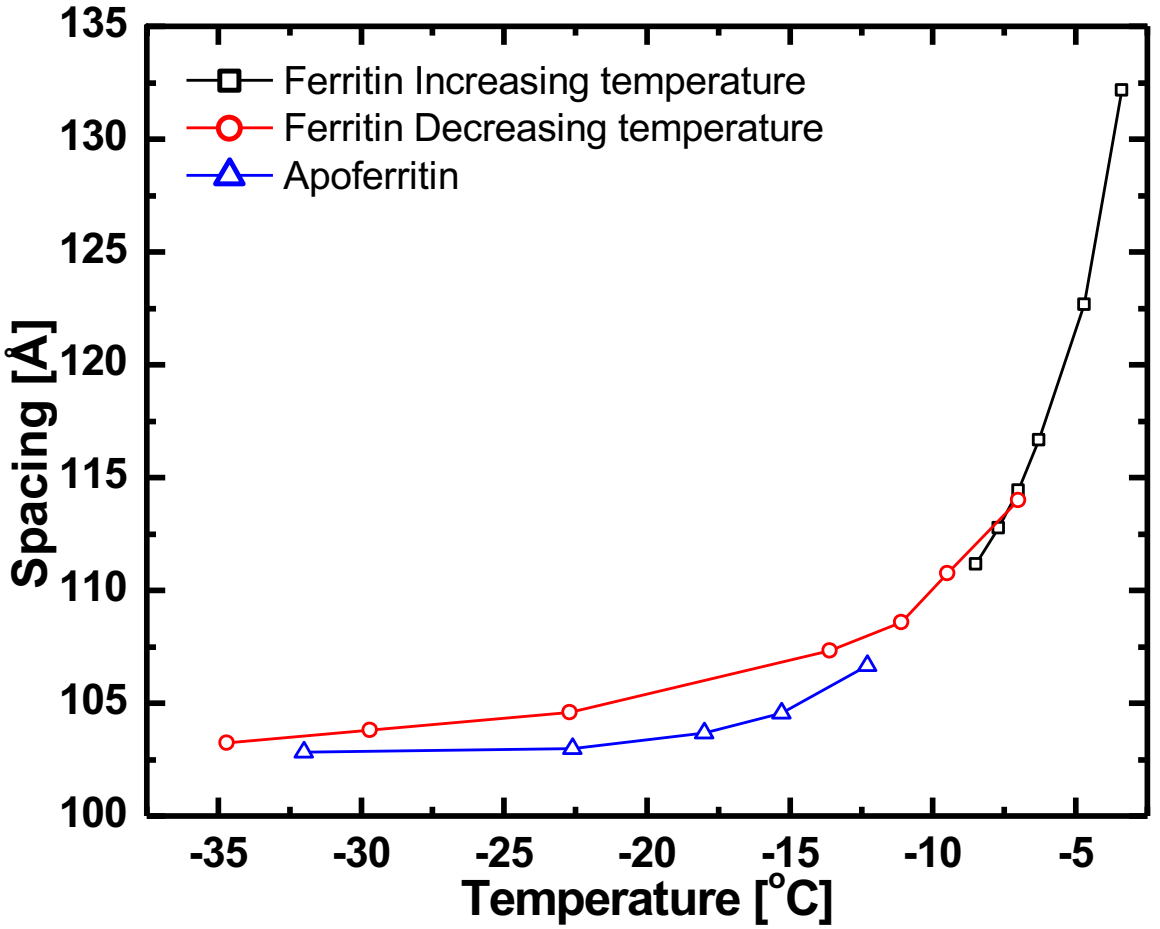
50 mg/ml



Thermal "expansion" is still present

Form factor (Bessel function) divided out

Apoferritin Thermal Expansion



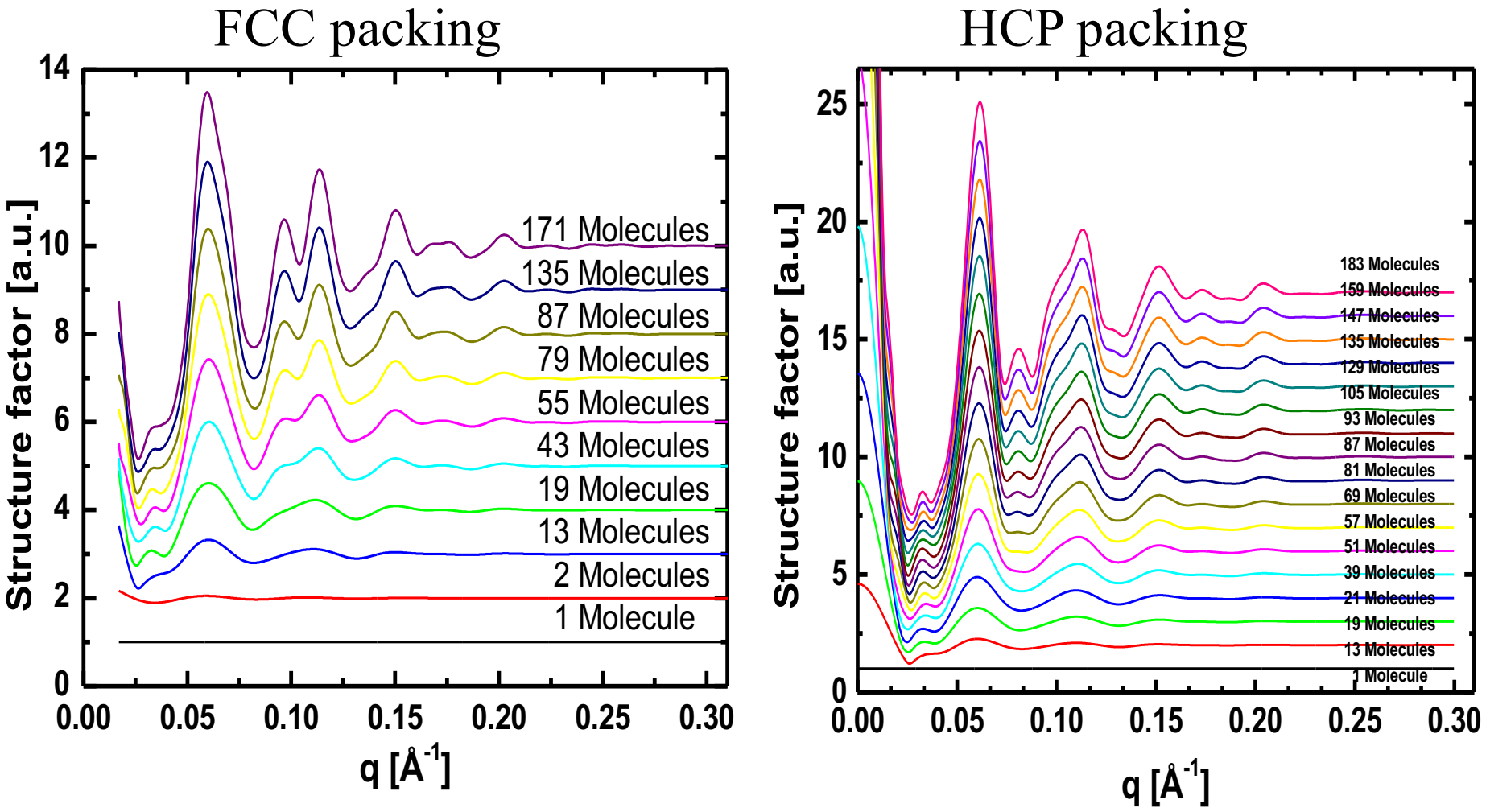
- The thermal expansion of apoferritin matches that measured from holoferritin.

Cluster Simulations

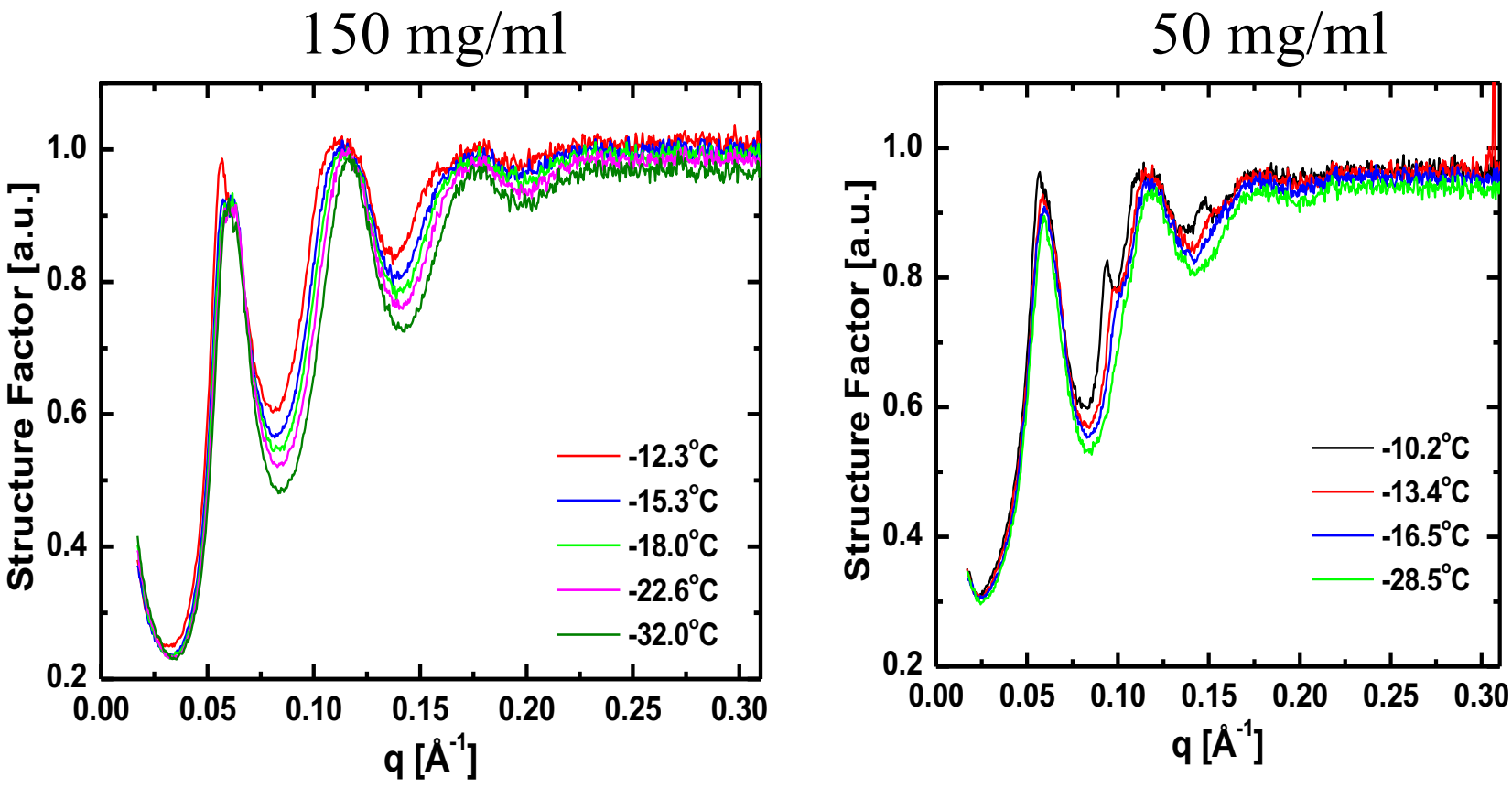
- We used the Debye formula to calculate the spherically averaged structure factor (S) of apoferritin clusters.
- Assume bulk crystal spacing.
- We add protein ‘atoms’ in symmetrical shells around a central molecule and calculate S:

$$S^2(q) = \sum_i^N \sum_j^N F_i F_j \frac{\sin qr_{ij}}{qr_{ij}}$$

Close-Packed Clusters



Apoferritin Structure Factors

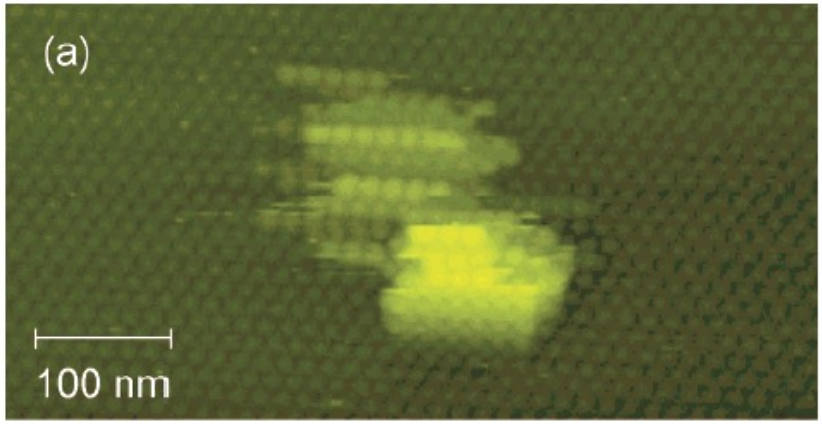


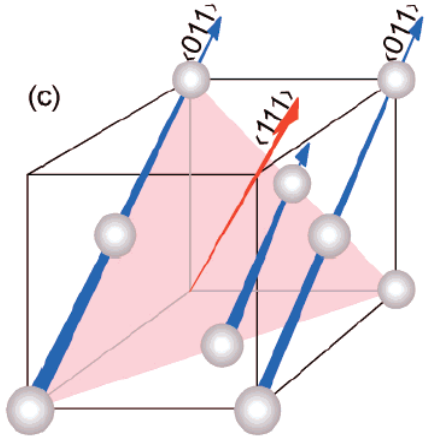
Thermal "expansion" is still present

Planar Ferritin Nucleation

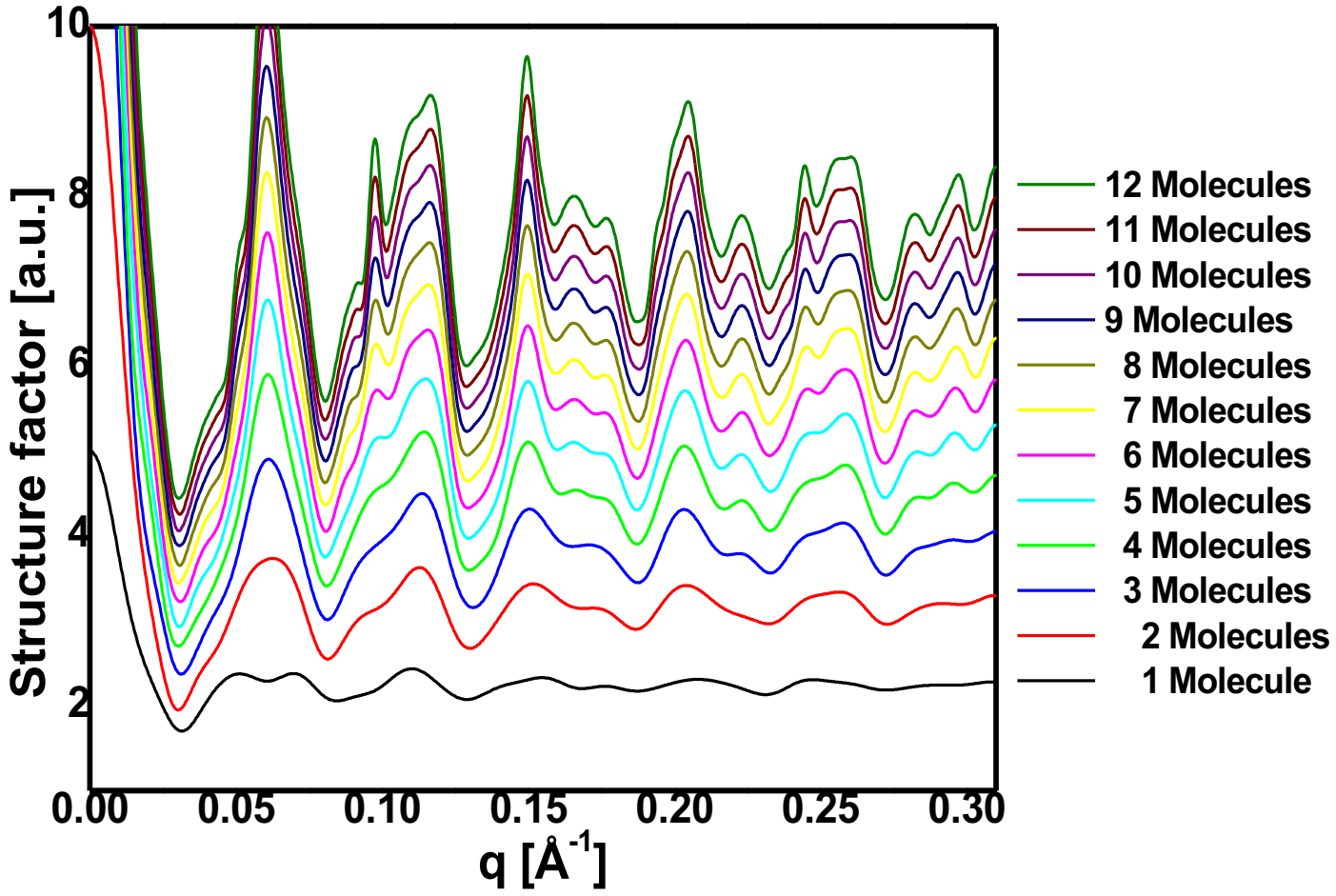
Yau S.-T. and Vekilov P.G., *J. Am. Chem. Soc.* (**2001**), *123*, 1080-1089

- Yau and Vekilov observed by AFM the formation of planar clusters of ferritin made of 4-7 <110> rods of 4-8 molecules.



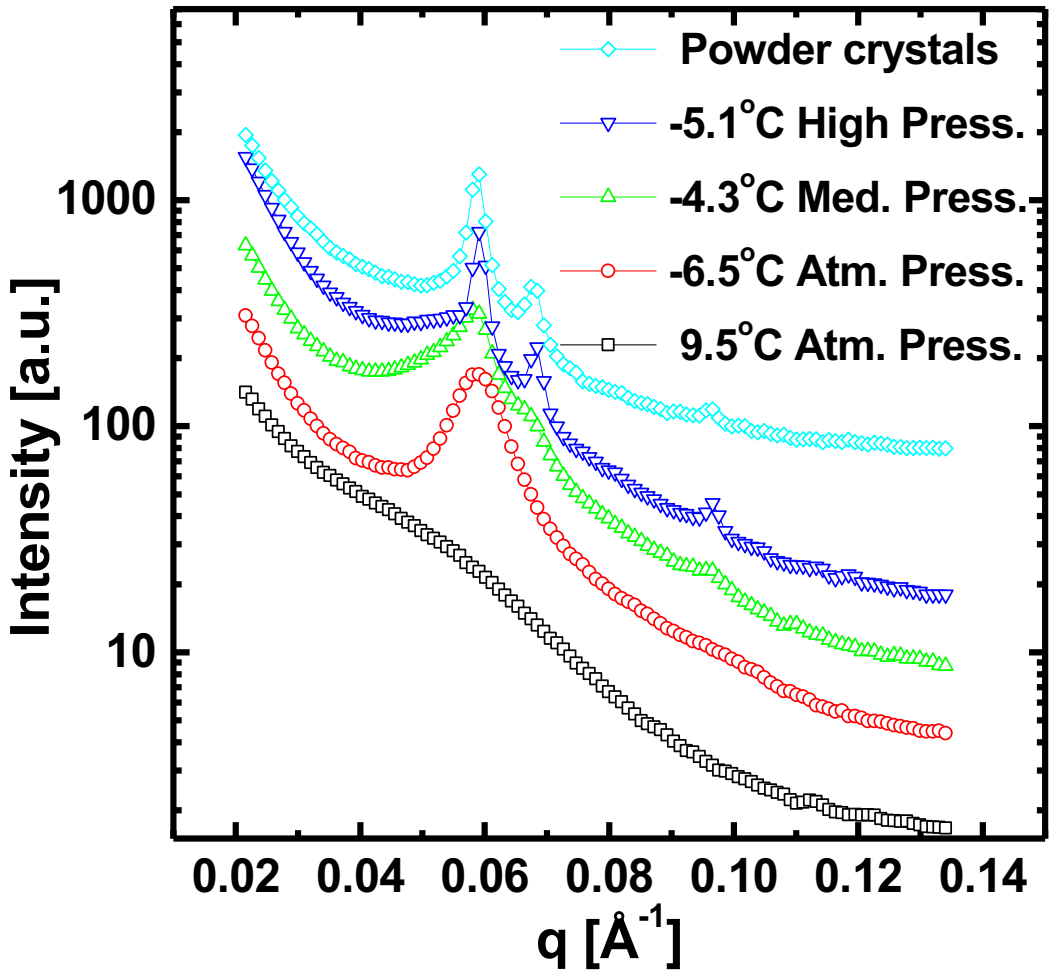


Planar clusters Simulation



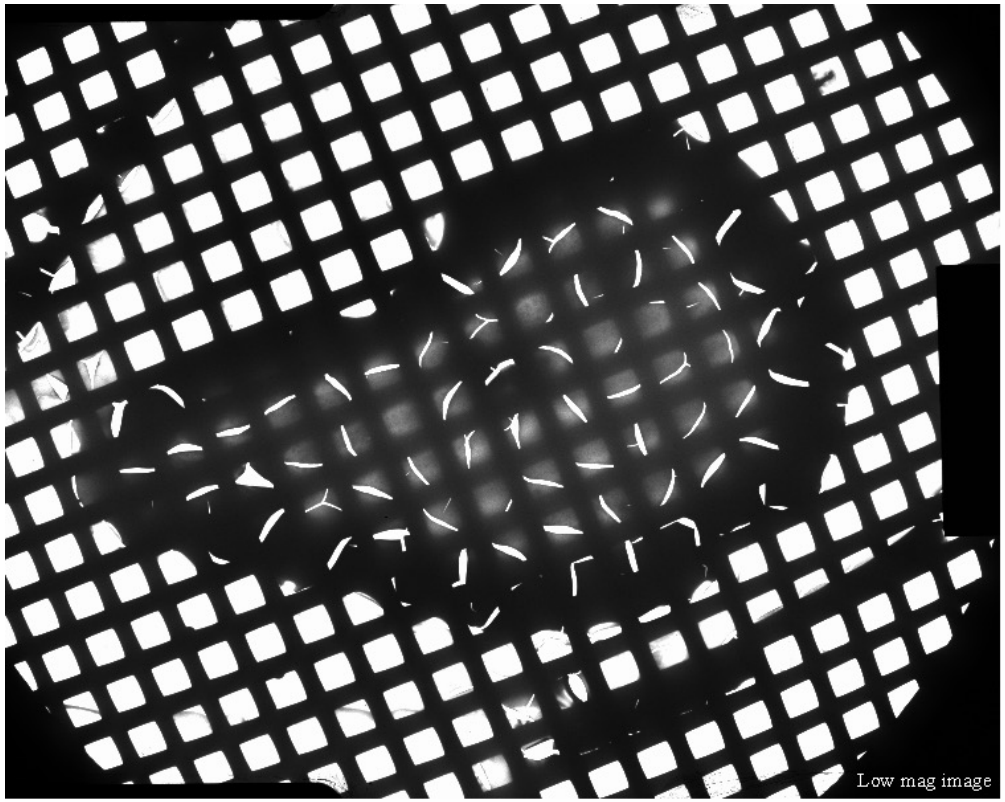
- Simulation of structure factor from clusters of 5 <110> rods of 1 to 12 molecules per rod

Hydrostatic Pressure



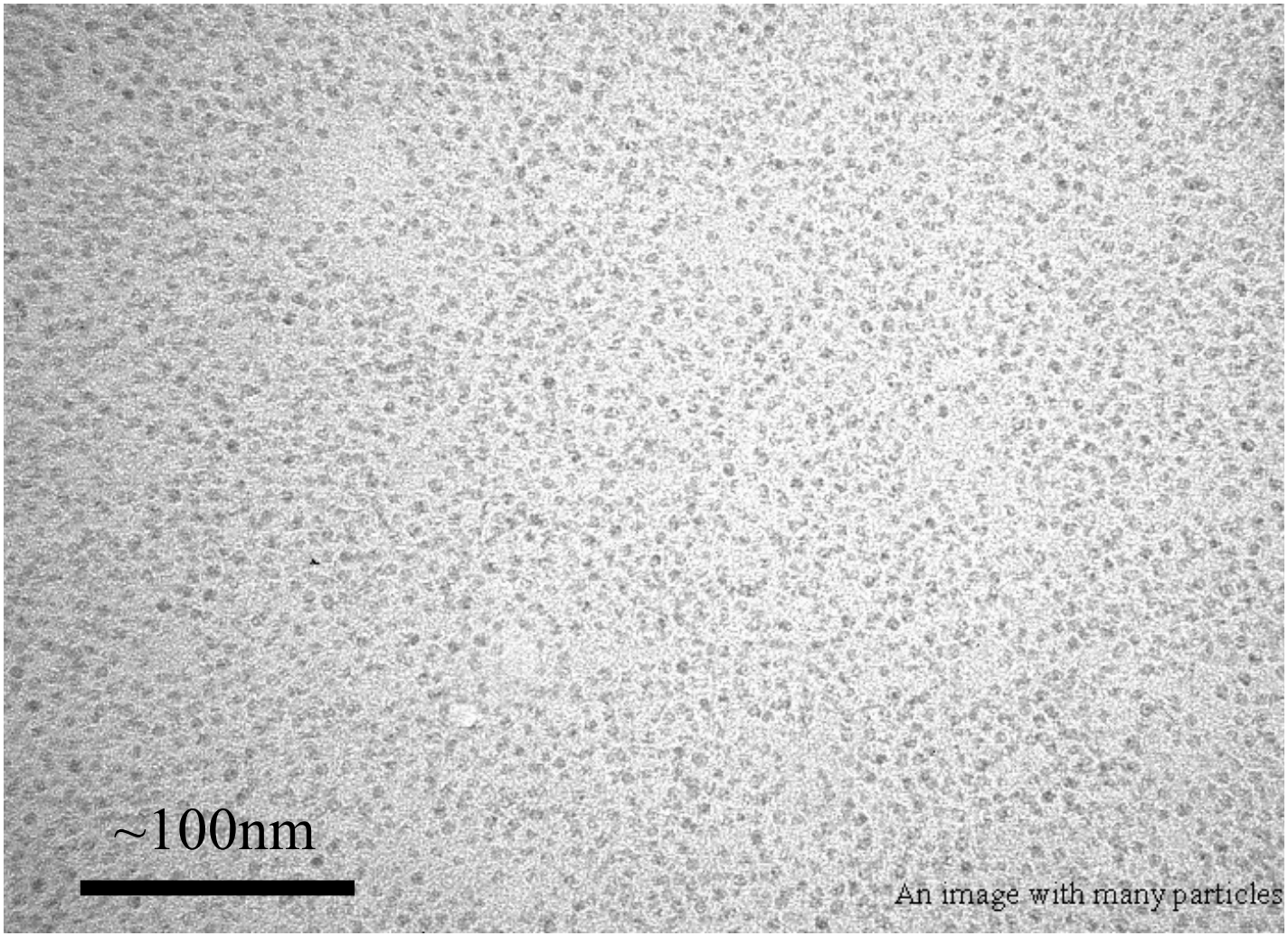
- Pressure generator glued to a sample capillary.
- Pressure transforms the broad peak into sharp Bragg peaks similar to those produced with Cadmium salt.

Overview of EM grid

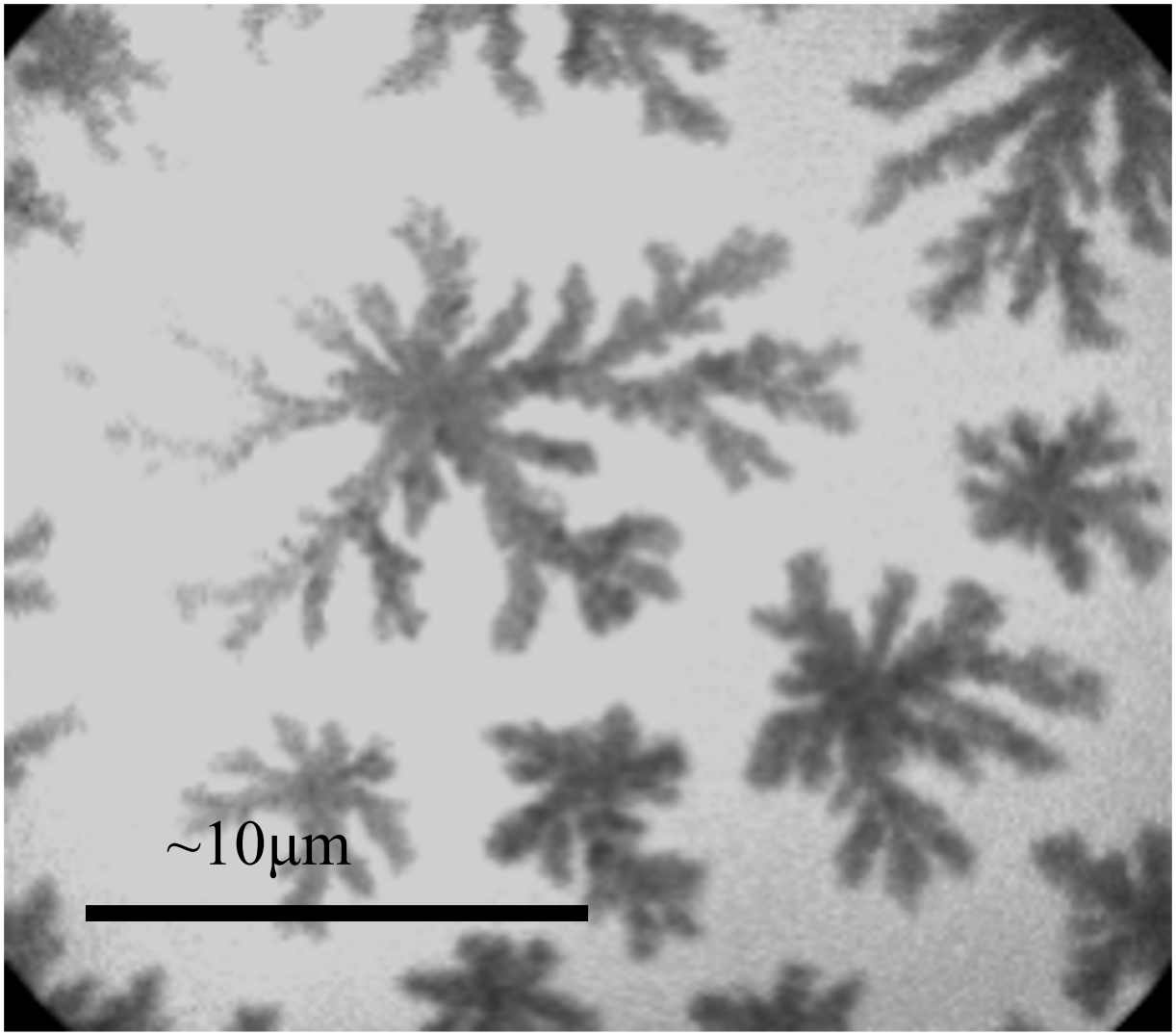


- 3mm diam grid
- a-carbon + "Formvar"
- 10mg/ml Ferritin
- 10mg/ml NaCl
- dipped grid
- dried in air
- no cryo-cooling
  - ▷three regions of different density

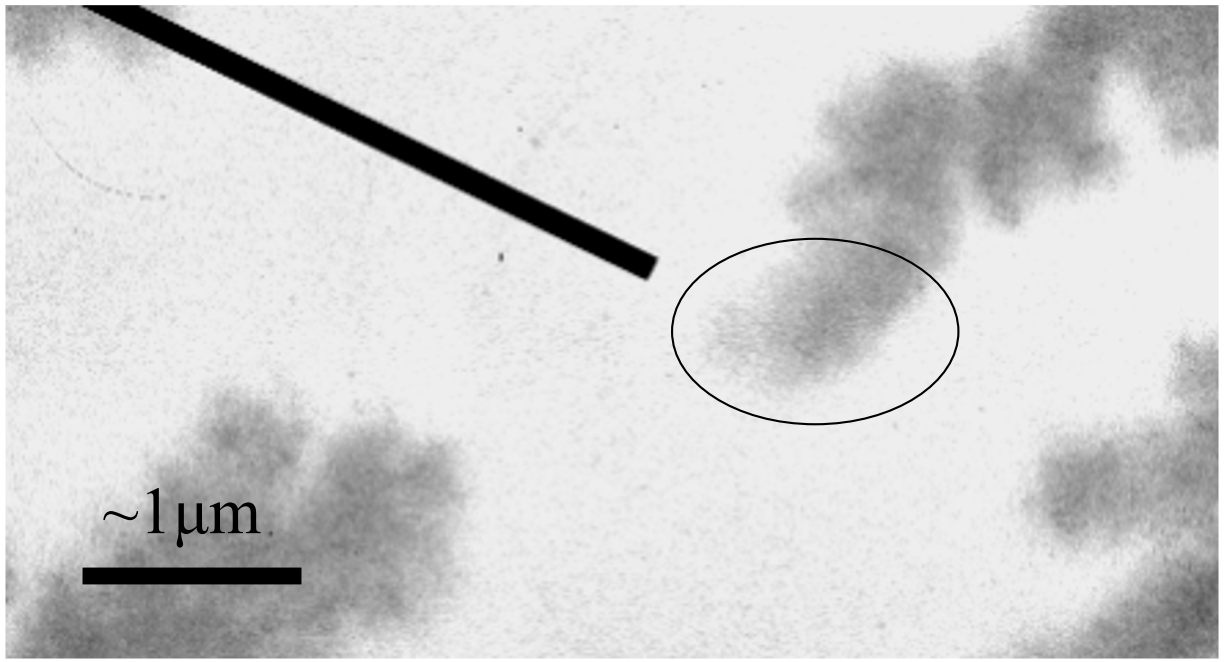
Border Region (low density)



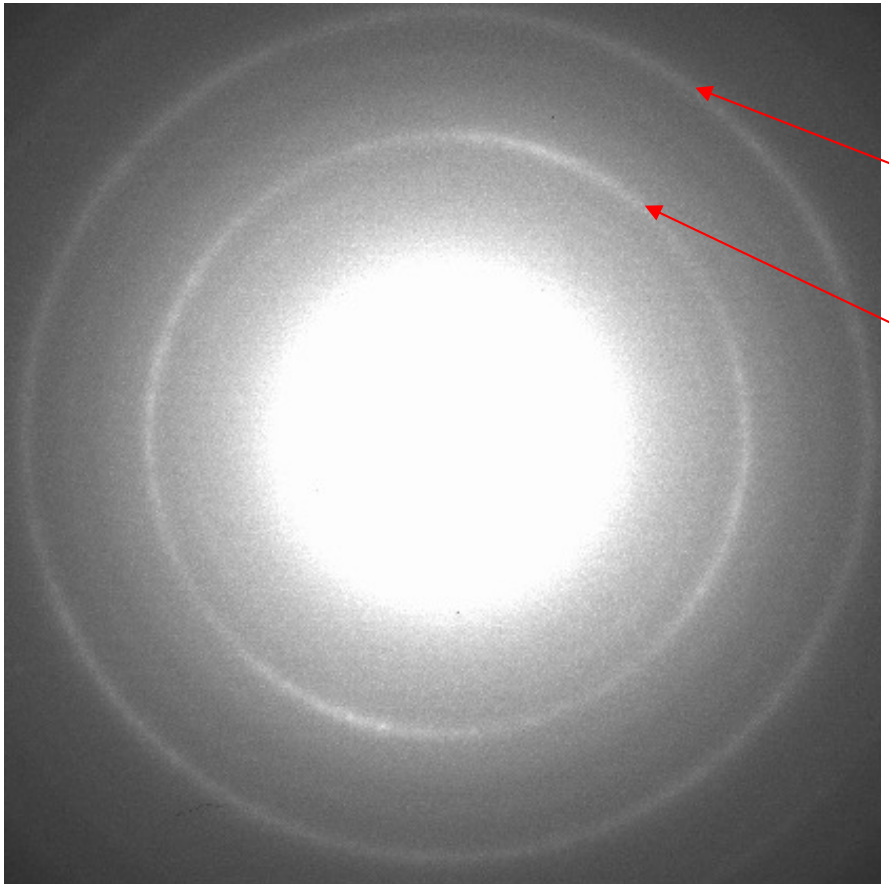
High Density Ring



Select Area around Dendrimer Tip



Selective Area Diffraction



$Q=6.32Å^{-1}$

$Q=4.44Å^{-1}$

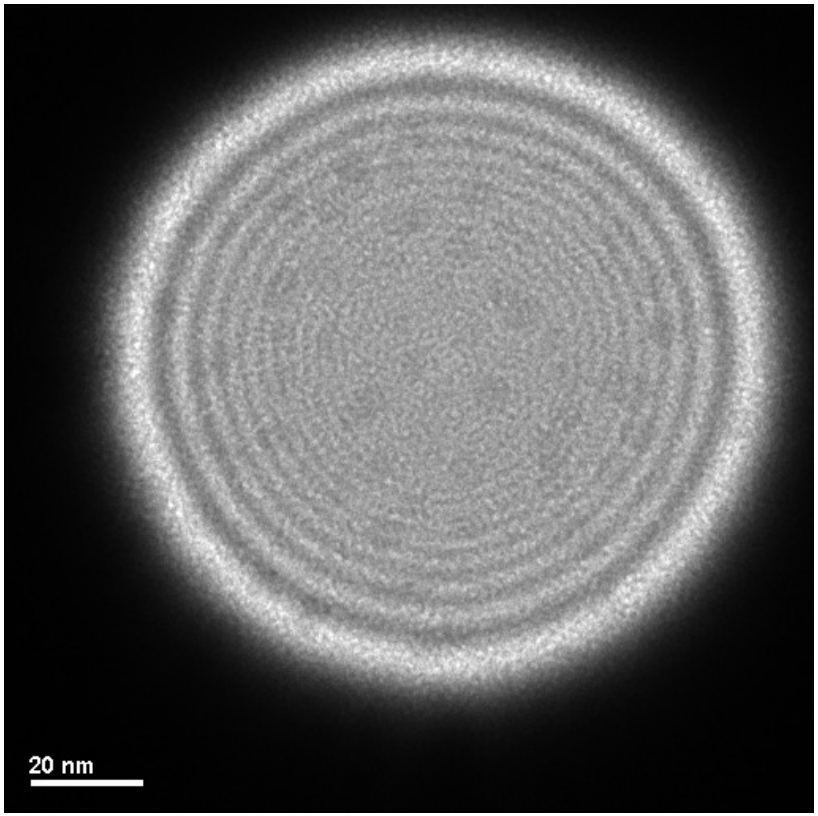
$\lambda = 0.025Å$

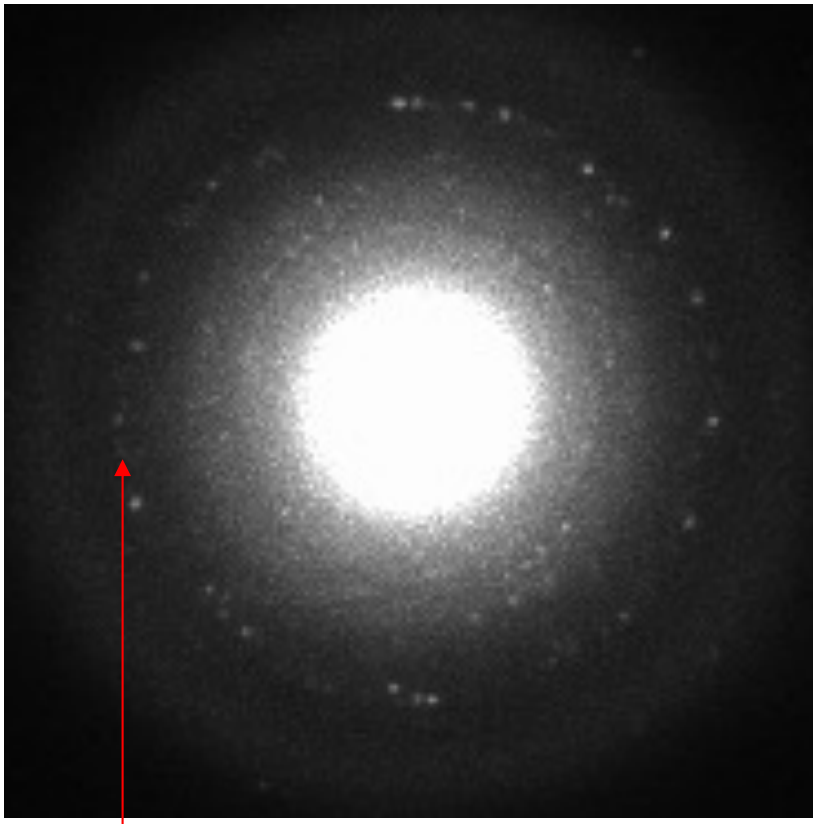
$D = 2.0m$

$R_1=35.4mm$

$R_2=50.4mm$

Diffraction from Border Region

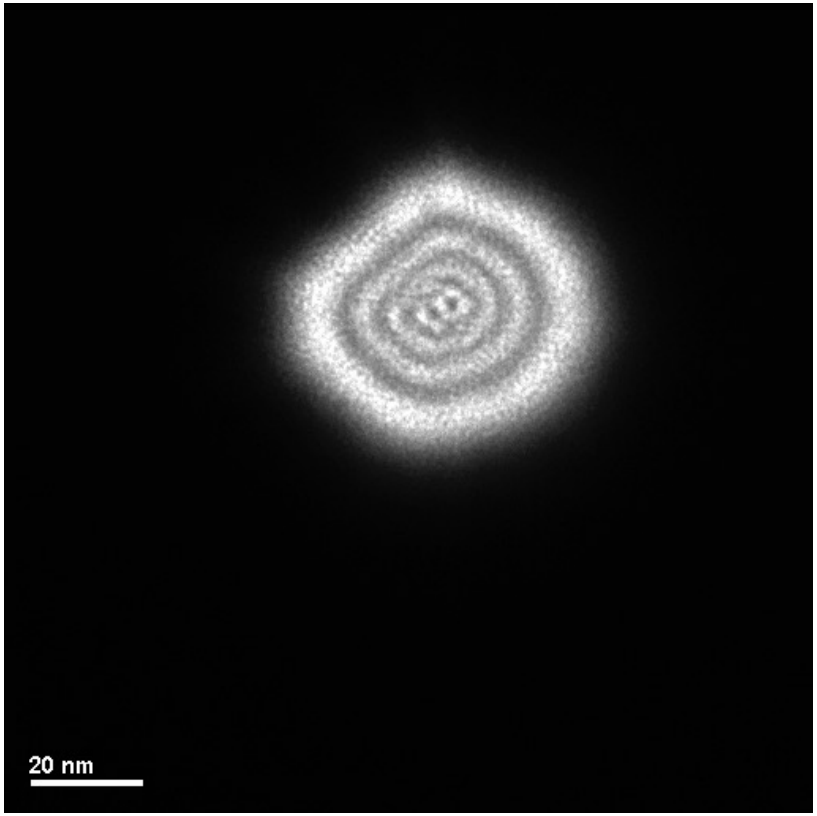


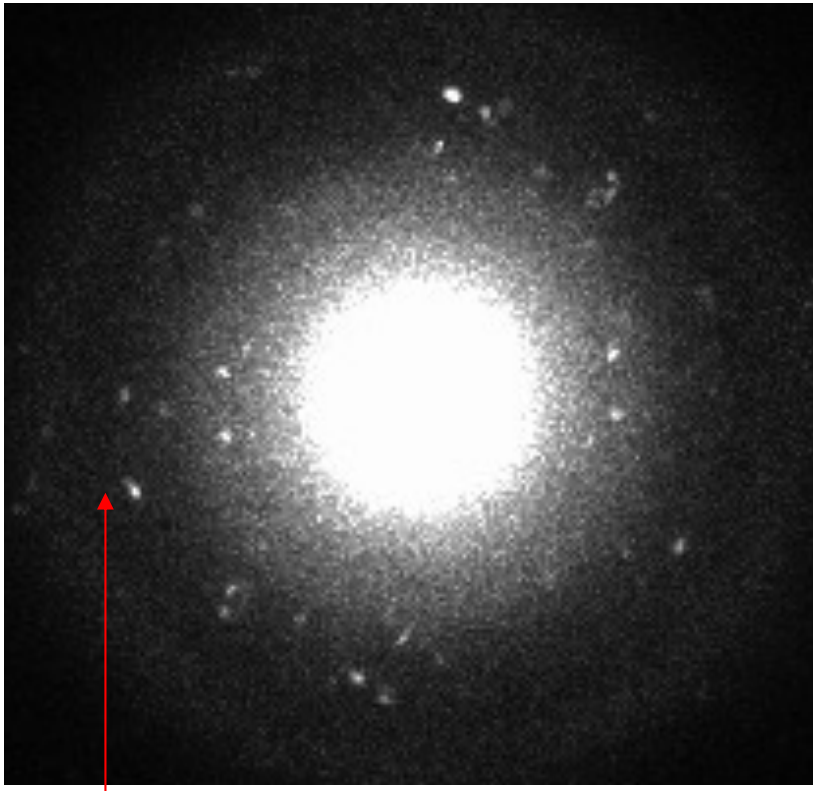


$Q=8.43 Å^{-1}$

Smaller Area Diffraction

Images: picture2 and diffraction4





Q=8.2Å^{-1}

Conclusions

- Smallest Ferritin crystals about 3 microns
- Implosion upon radiation damage
- New condensed state upon freezing
- SAXS consistent with planar or 3D cluster
- Large thermal expansion of cluster
- Single molecule diffraction ideas can be tested by electron diffraction