Macromolecular Crystallography at Synchrotrons

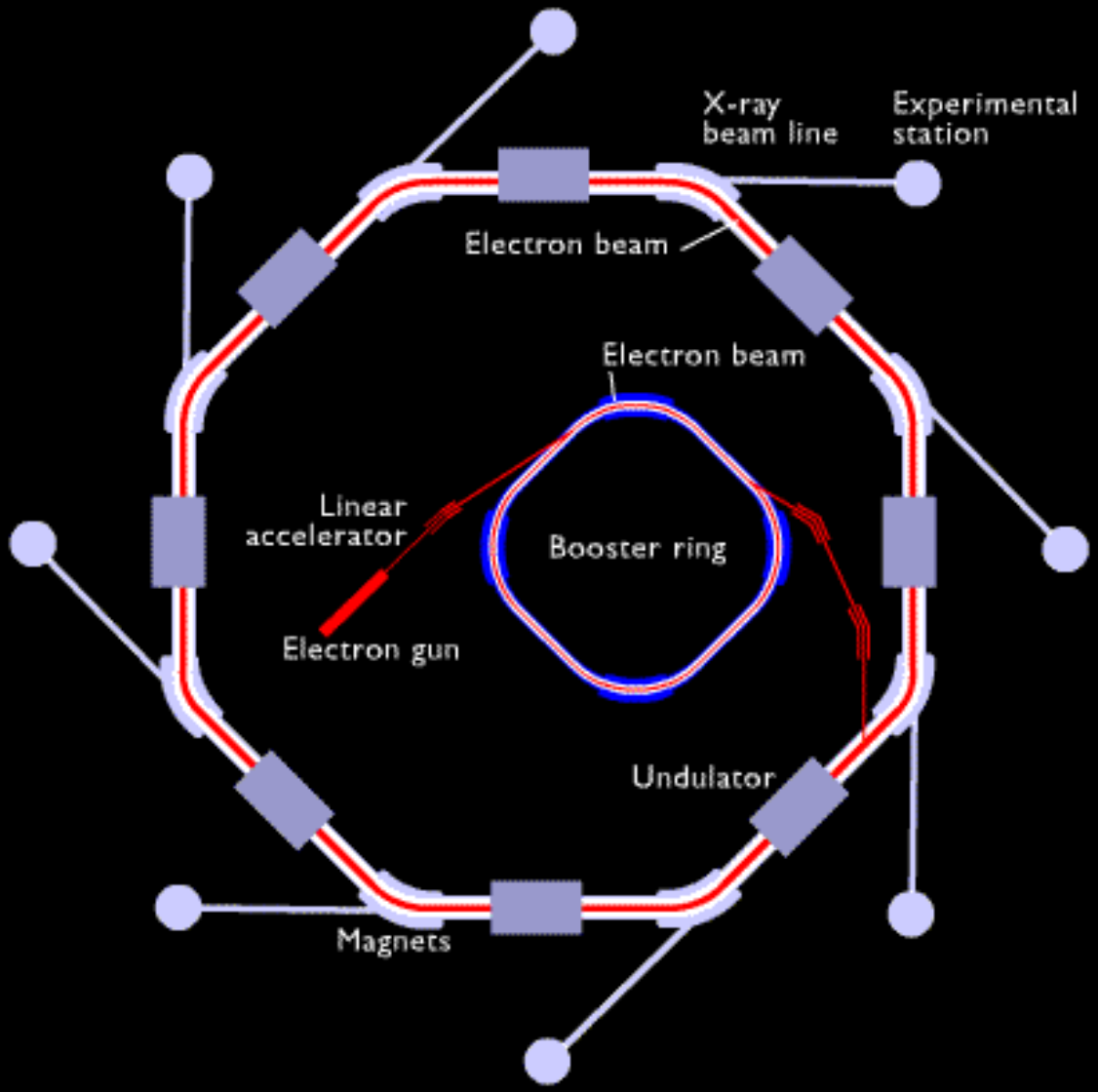
A synchrotron accelerates and stores particles (electrons or protons) moving at speeds close to that of light.

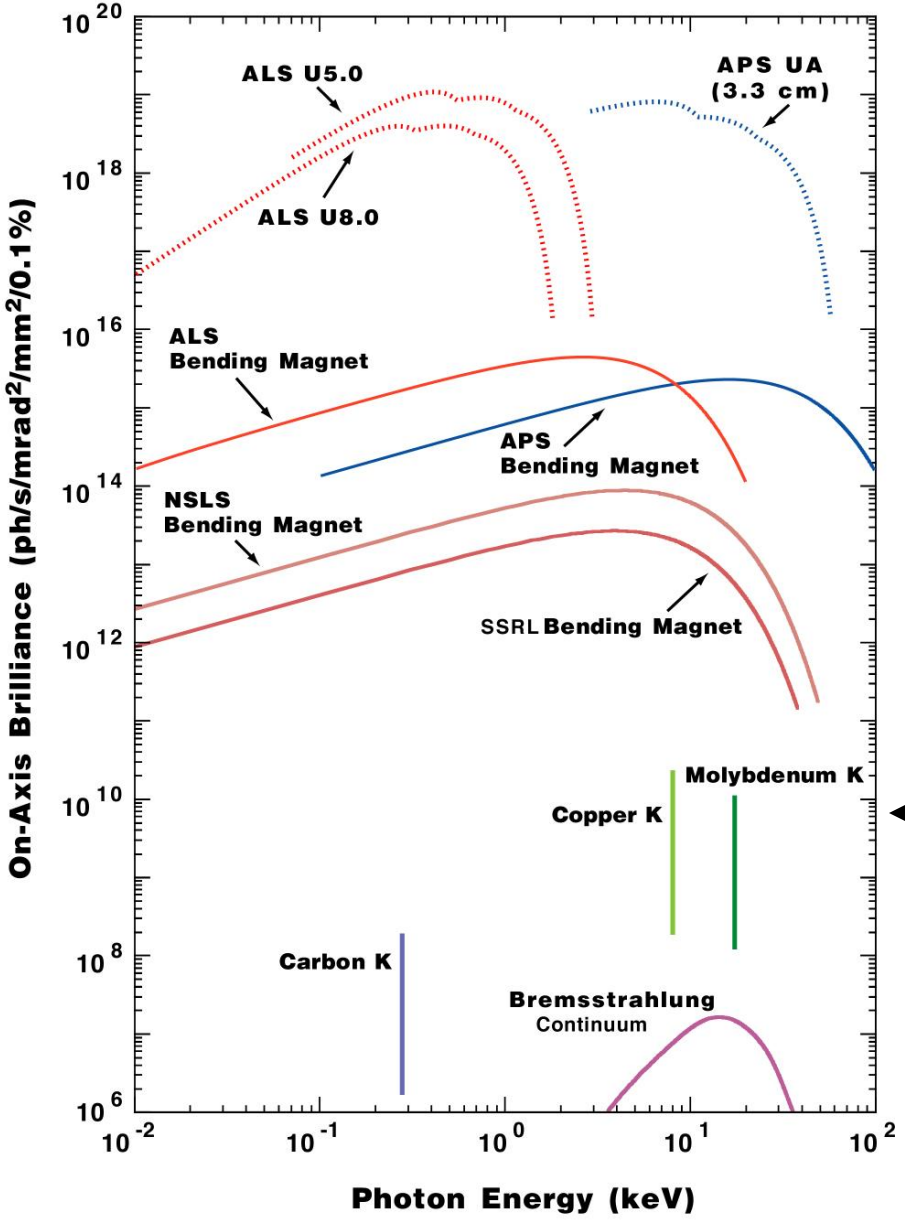
As the particles loose energy they give of electromagnetic radiation.

The particles are steered by magnetic fields.

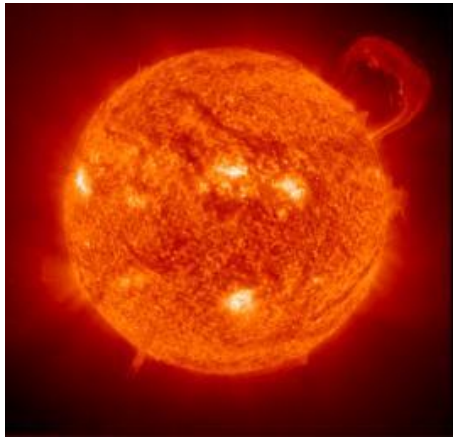
Electromagnetic radiation (photons) is not affected by these fields and is emitted at the tangent to the change in direction.

Insertion devices (undulators and wigglers) ‘amplify’ this radiation





Synchrotron radiation is 10^9 times
More brilliant than the sun
and about 100 million miles closer



Why use a synchrotron?

- Roughly (very roughly) - The signal to noise in the data goes up by the log of the increase in brilliance.
- Brilliance has units of photons per second, per $mrad^2$ per mm^2 per 0.1% relative bandwidth.
- Laboratory X-ray source, brilliance $1.0x10^{10}$, synchrotron $1.0x10^{18}$, $Log(1.0x10^8)=8$ fold potential increase in signal to noise.

Another area of research saved for a later date – *i.e.* What will my crystal diffract to if it diffracts to X Å at home?

Potential pitfalls?

- Heat and radiation.
  - Is heat a problem that could be addressed to help improve data?
  - Radiation damage is known to be a problem, what causes the damage, how is it manifested, can we reduce it or even use it?

Caution – Work in progress (Raw Data)

Outline of talk

- Beam heating
  - Modeling and measuring beam heating
- Radiation damage
  - Process of radiation damage
  - Henderson limit
  - Practical limits
  - Experiment
  - Results
  - Can we reduce or prevent the damage
  - Can we make use of the damage

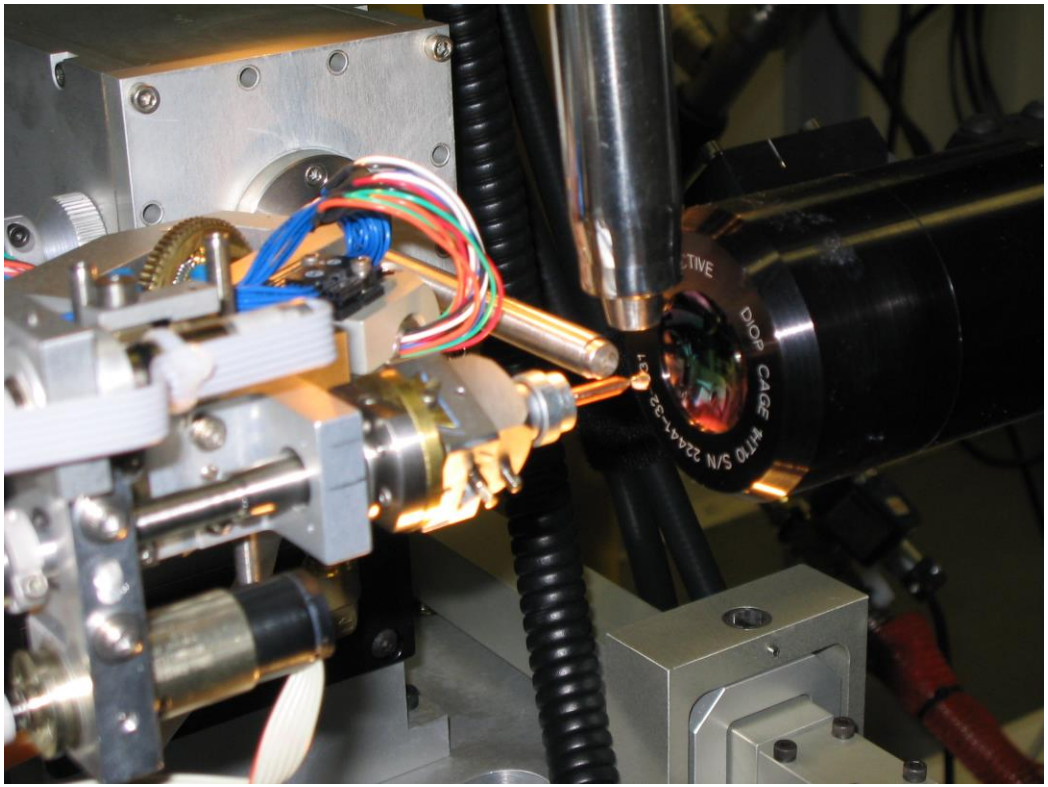
Beam Heating

- How much heat does an intense synchrotron beam deposit on a crystal?
- Does the cryostream effectively take this heat away?

To date

- Steady state data processing and modeling almost complete (paper in preparation).
- Time resolved analysis starting

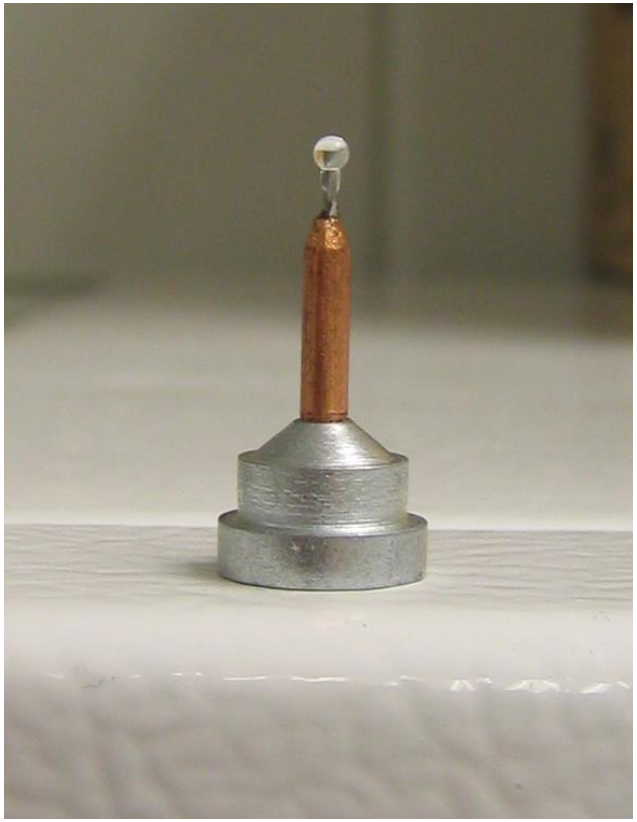
Experimental



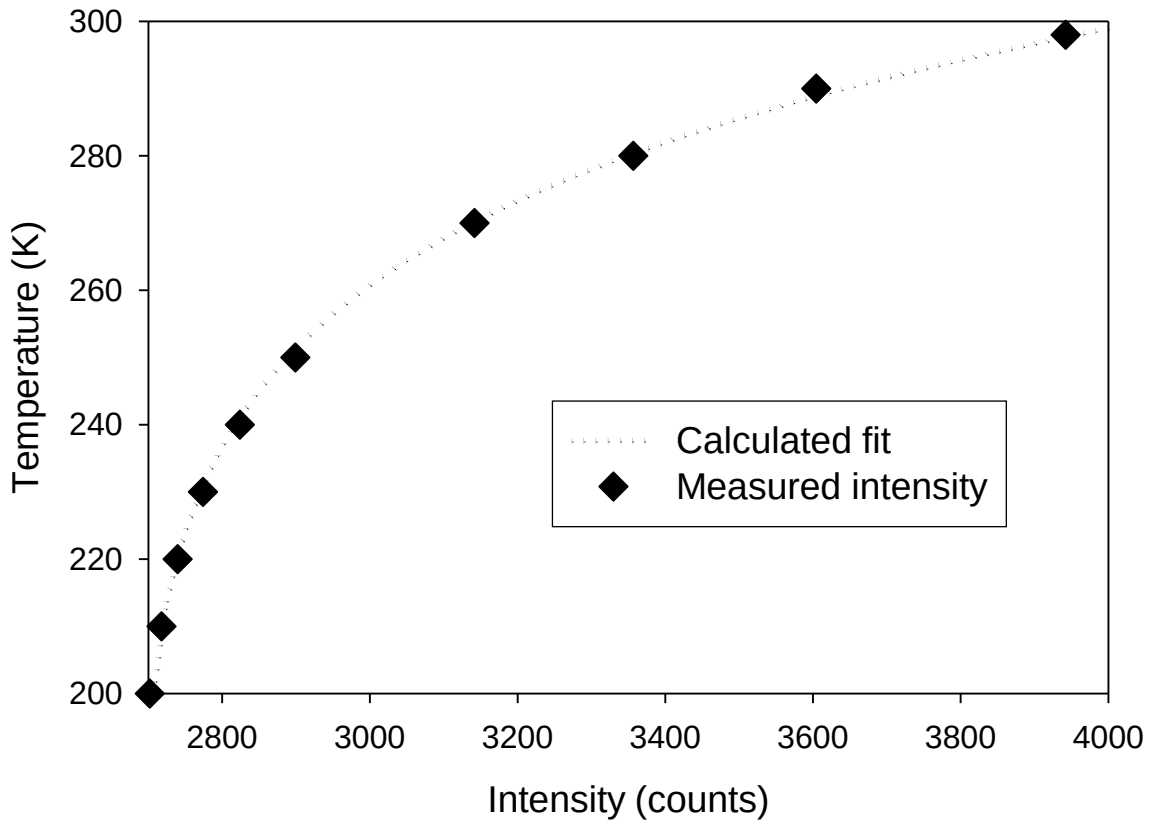
- Advanced Photon Source beamline 19-ID Structural Biology Cat
- Oxford 700 cryostream used to cool sample
- Samples imaged with thermal imaging camera

Glass Bead Samples and Protocol

- Sample 1: 2mm diameter glass bead
  - imaged at 100K with no beam (steady state calibration point)
  - Imaged with shutter opening (time resolved)
  - Imaged after shutter had been open for 1 minute (steady state).
  - Measurements repeated in 10K steps up to 290K
  - Final measurement with the cryostream off.
- Sample 2: 1 mm diameter glass bead
  - Imaged from 290K down to 100K in reverse of 2mm case.

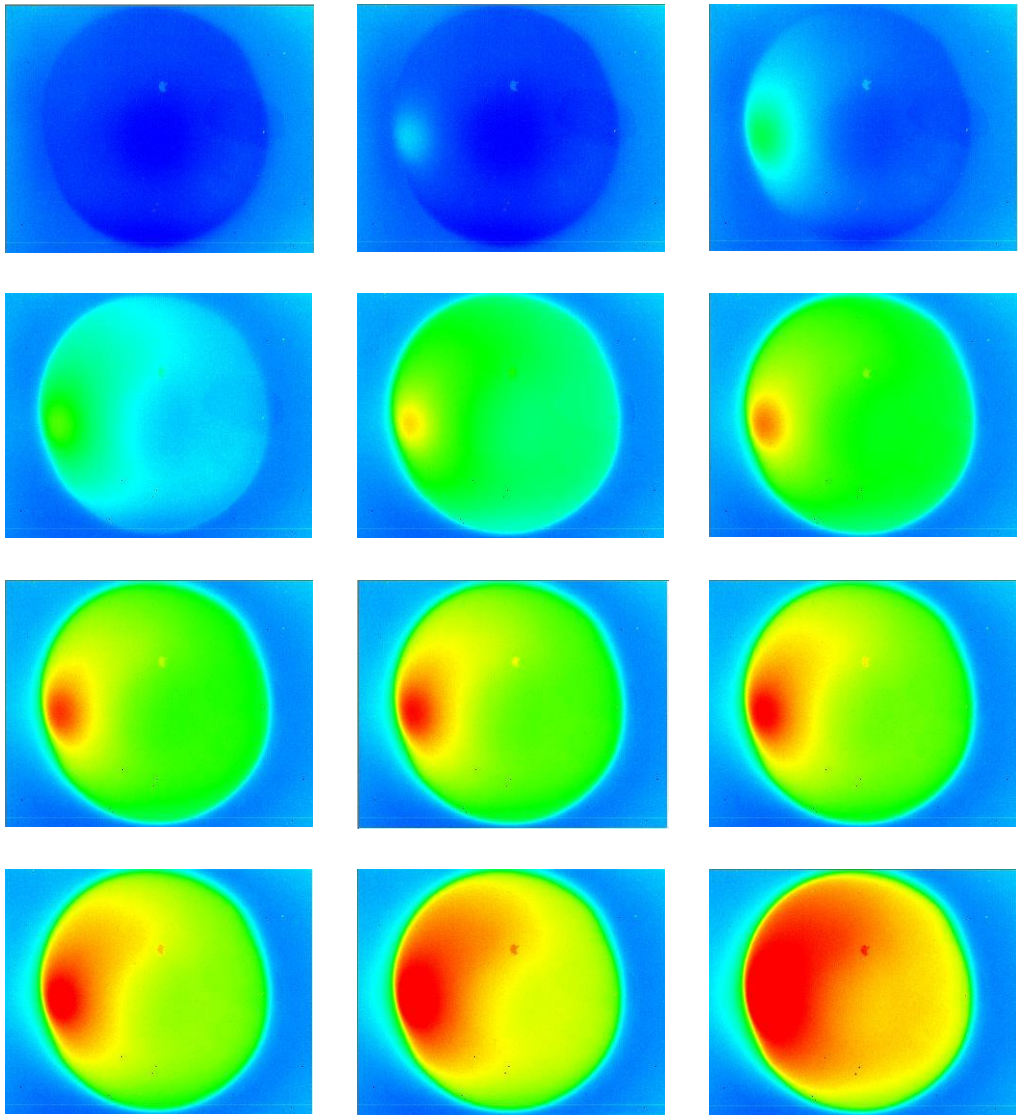


Temperature calibration



Bead at known temperature when shutter is closed. The intensity determined at this temperature and a calibration curve of intensity versus temperature calculated.

Shutter Opening

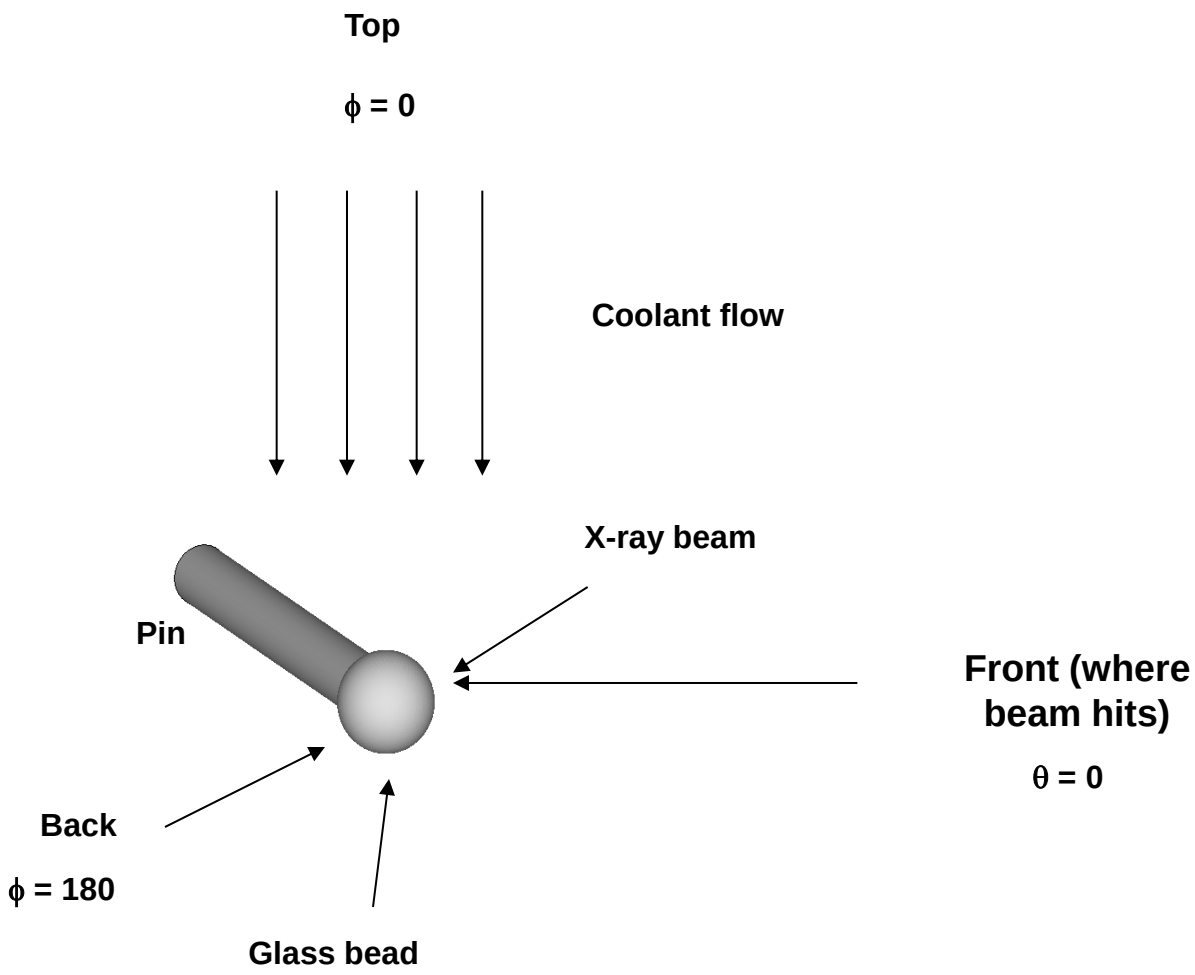


2mm glass bead at room temperature with no cryostream flow.

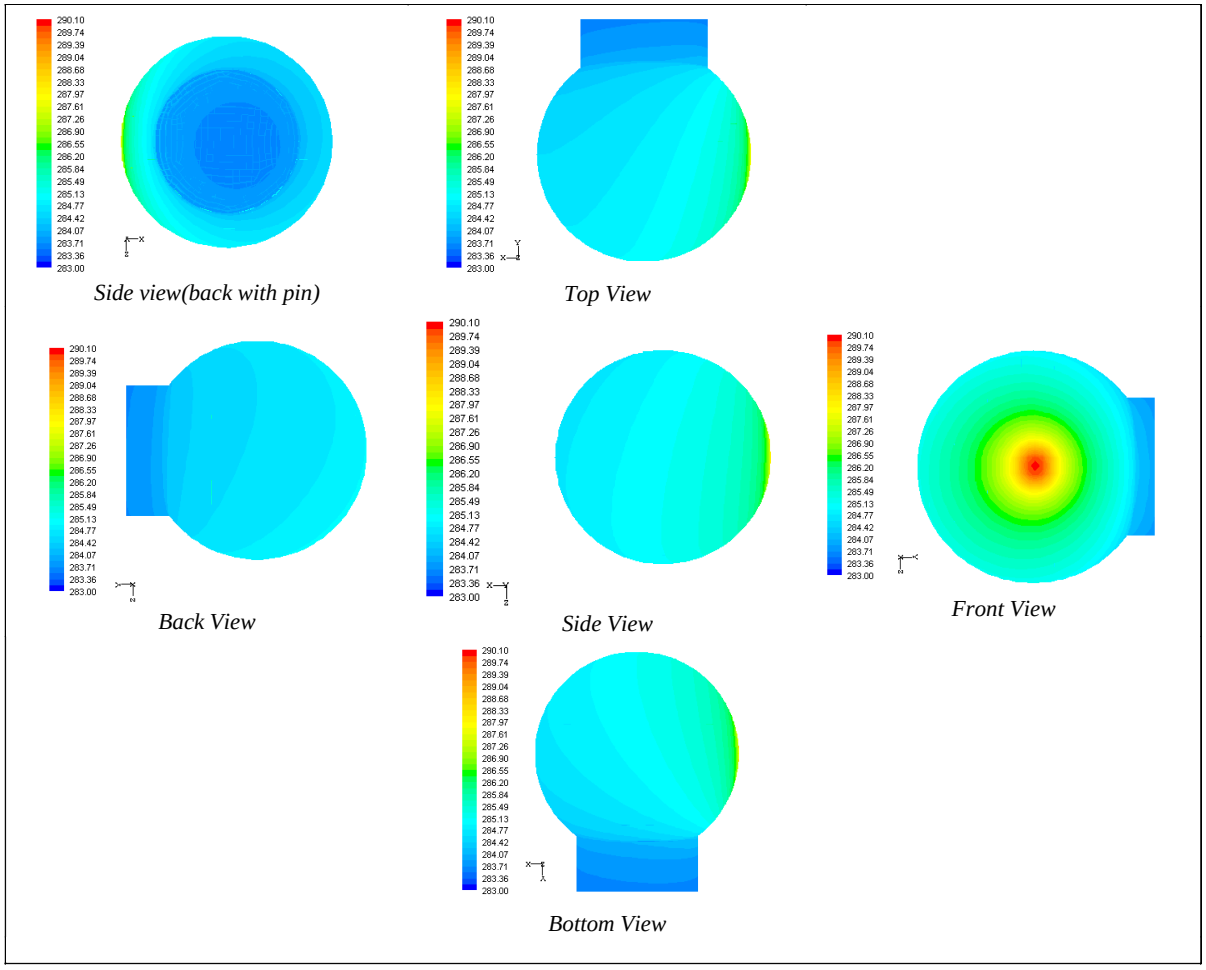
Beam hits bead from left, blue is cold, red is hot.

Temperature rise is 50K over 24 seconds.

Extreme case!


Top
ϕ = 0
Coolant flow
X-ray beam
Pin
Front (where beam hits)
θ = 0
Back
ϕ = 180
Glass bead

Model

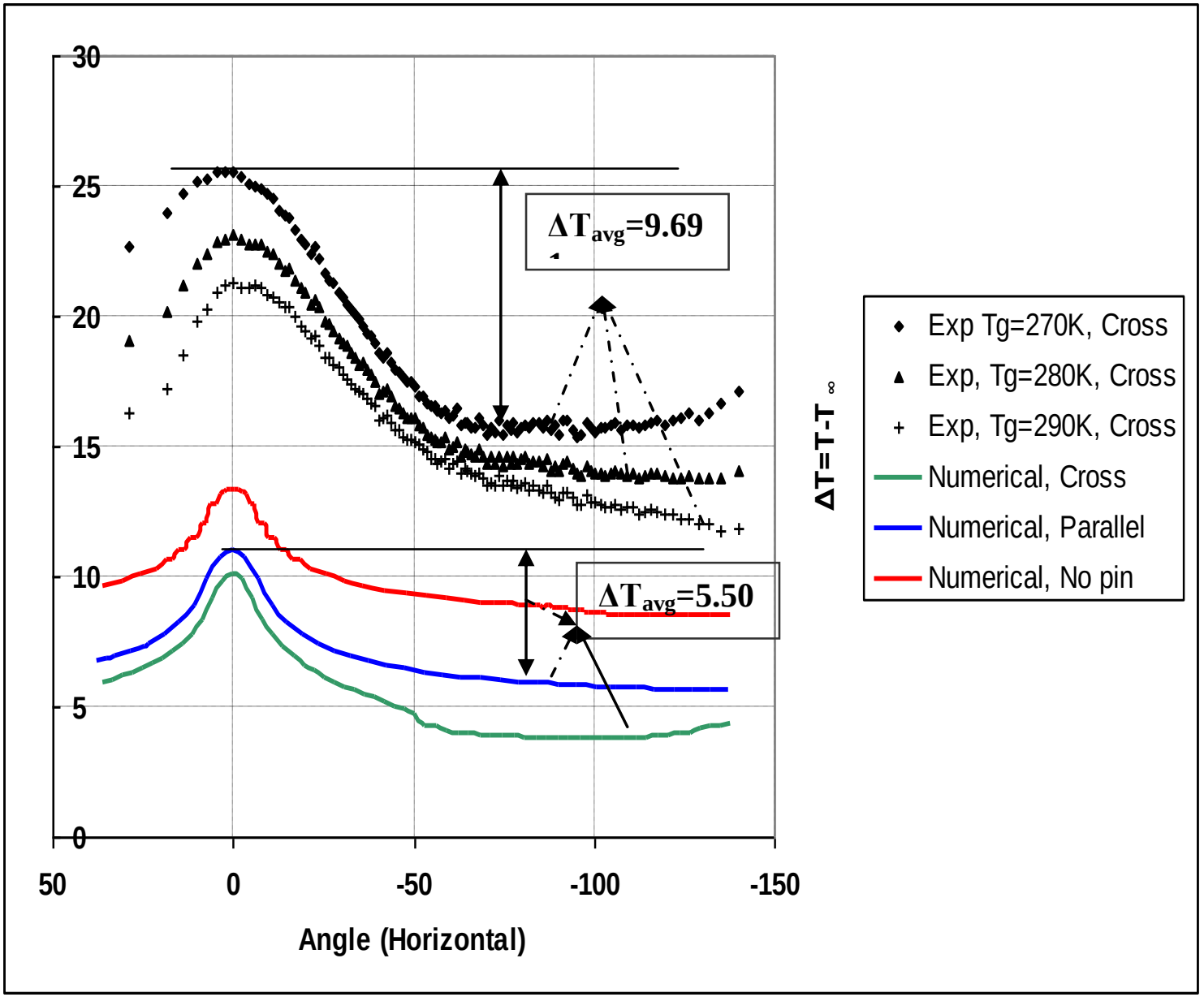


Model uses finite element analysis and fluid dynamics.

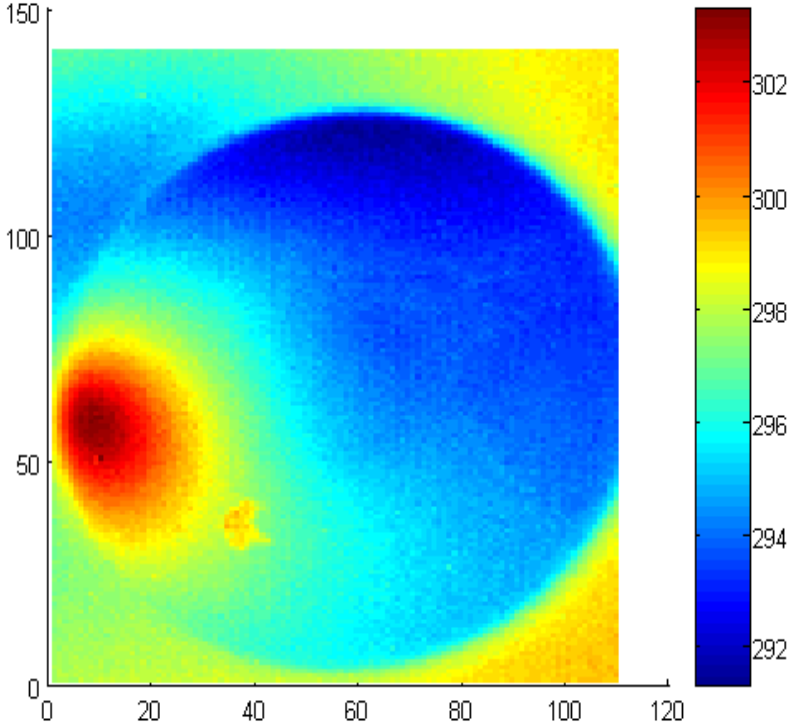
Any portion of the glass bead can be examined.

Model is currently steady state. i.e. constant heat load and constant cooling.

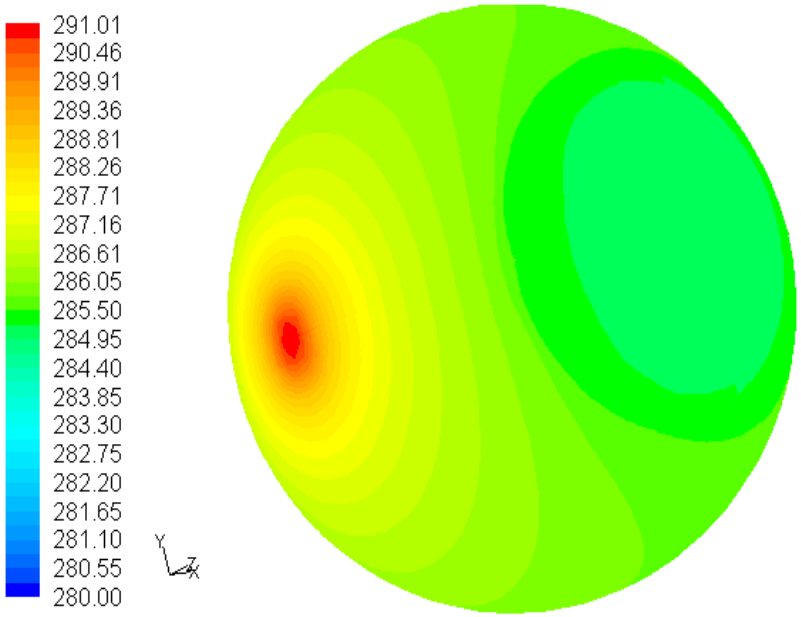
Model versus experimental



Model versus experimental



Experiment



Model

Other work in process

- Time resolved heating:
  - Initial results are from a steady state model, a time resolved model is in development.
- Other samples: Real protein crystals:
  - Data also collected on real protein crystals and loops with liquid in them.
  - Radiation caused changes in the infrared emittance properties (this means structural properties are changing).
  - Subsequent data collection using infrared laser to put same heat load as beam on sample.
- Flow rate
  - Data collected on different flow rates.
- Thermocouple
  - Data collected on thermocouples in the cryostream

Summary

- Heating does happen.
- Phase change (occurring at ~140K for ice) is not a problem, even in this worst case scenario.
- Heating may cause small cell parameter changes.
- Systematic error in theory, about 5K difference in results.
- Suspected cause of error now known, incident beam calculations are being recalculated.
- No other model this accurate exists to date
- Model can be used to look at different heat transfer methods, e.g. flow, cryogen, pulsed exposure etc.

Why are we doing this

- To produce an accurate model that can be used to design improved cryopreservation techniques, model changes in diffraction data over time and provide data for in silico modeling of the process a crystal goes through when in the beam.

Acknowledgements

- University of Cincinnati – Computational modeling and theory development
  - Mike Kazmierczak
  - Pradeep Gopalakrishnan
  - Raghav Sampath
- Advanced Photon Source – Beamline operations and flux calculations
  - Gerd Rosenbaum
- Marshall Space Flight Center – Data collection
  - Mark van der Woerd
- CAMD – Data collection
  - Henry Bellamy

- Staff at SER-CAT and SBC-CAT

Radiation Damage

- How is radiation damage manifested in X-ray data?
- Are there any metrics that can be used to measure it?
- What are the effects on a structural level?

To date

- High resolution radiation damage data sets collected from xylose isomerase.
- Data processed and signal-to-noise and cell parameter effects noticed.
- Structural effects also seem to be occuring.

Radiation Damage

- 1 Å X-ray interaction in a crystal
  - 90% of the X-rays pass straight through (the reason for the beam stop).
  - 8.4% interact by the photoelectric effect. All the X-ray energy is transferred to an electron which is then ejected (main process of radiation damage).
  - 0.8% interact through Compton scattering. The X-ray transfers some of its energy to an atomic electron and a second lower energy photon is released. This forms the incoherent background.
  - 0.8% interact through Thomson (Rayleigh) scattering elastically with no energy loss. This is the X-ray that gives diffraction data.

Processes of radiation damage

Primary, secondary, direct and indirect radiation-damage events in a protein crystal.

The incoming X-ray photons cause primary damage events, represented by darker stars. The paths of secondary radicals are shown by dotted arrows, and the damage events they induce are represented by lighter stars. Direct events occur on the protein molecules, and indirect events occur in the solvent region.

Primary effects are a fact of life, we cannot prevent them. Secondary effects are reduced by cryocooling.

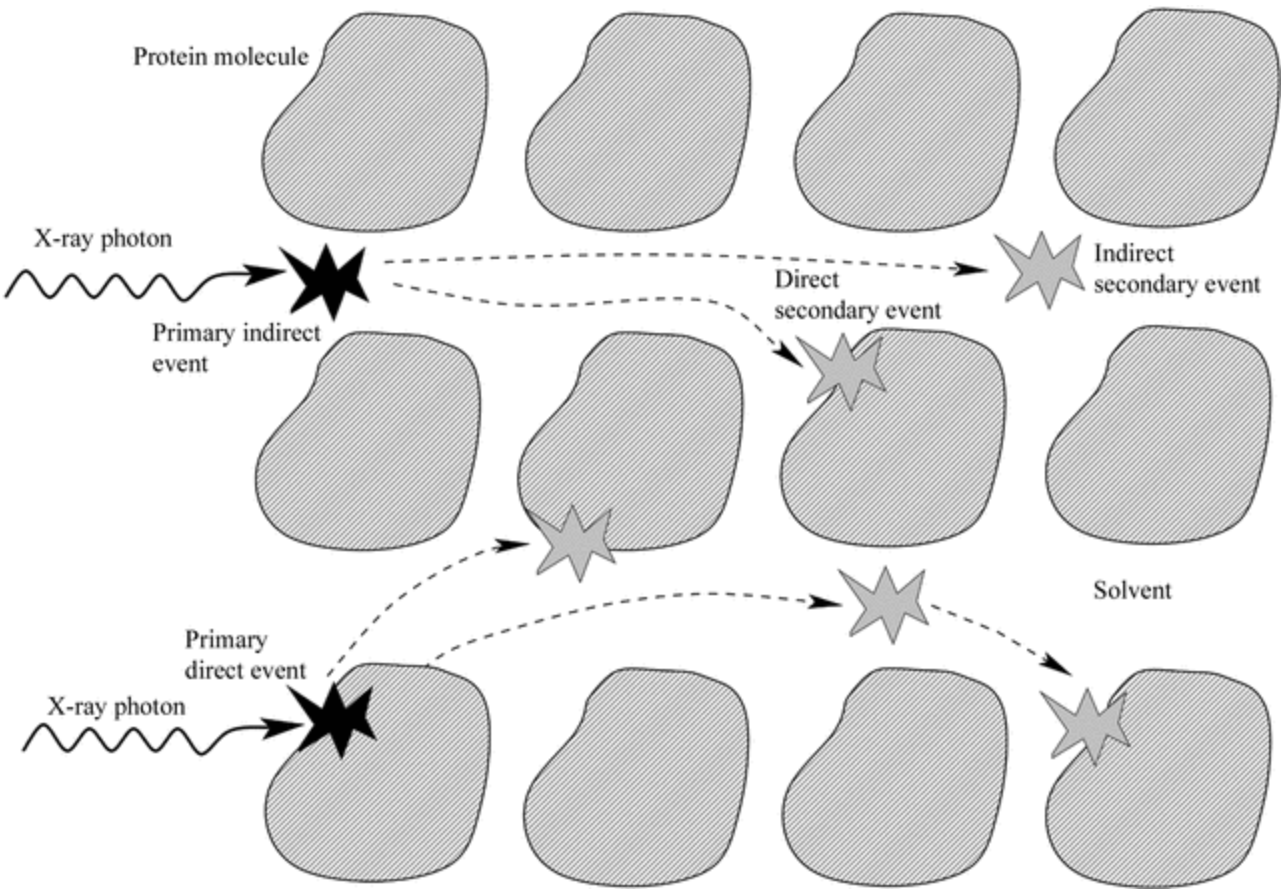


Image from Elspeth Garman

X-ray Radiation effect on water

Ionizing radiation can remove an electron from water:

$$H_2O^+ + H_2O \longrightarrow H_3O^+ + OH$$

And the ejected electron

$$e^- + H_2O \longrightarrow OH^- + OH$$

The simultaneous formation of H and OH free radicals gives further reactions

$$H + OH \longrightarrow H_2O$$

$$H + H \longrightarrow H_2$$

$$OH + OH \longrightarrow H_2O_2$$

Henderson Limit

- Radiation damage by electrons and X-rays are comparable.
- Electron diffraction patterns fade to ½ their original intensity after 1 electron $Å^{-1}$at room temperature or 5 electron $Å^{-1}$at 77K.
- The amount of energy absorbed per unit weight is expressed in units of gray (Gy). One gray dose is equivalent to one joule radiation energy absorbed per kilogram. One gray is equivalent to 100 rads.
- 5 electrons $Å^{-1}$ is approx $5x10^{7}$ Gy.
- The depth dose curve (maximum dose at ~100 μm) reduces the energy deposition so the effective energy causing the damage is conservatively $2x10^{7}$ Gy.
- X-rays of 1.5 Å give $12x10^{-16}$ Gy per photon m^{-2}.
- The X-ray flux giving rise to $2x10^{7}$ Grays is $1.6x10^{16}$ photons mm^{-2}

(Henderson (1990) Proc. R. Soc. Lond. B. 241, 6-8).

What does it mean practically: Dead Crystals

- Remember,
  - The X-ray flux giving rise to 2×10^7 Grays (dead crystals) is 1.6×10^{16} photons mm^{-2}
- Lab source – crystals at 77K (close enough to 100K)
  - 1×10^8 photons s^{-1} mm^{-2}
    - Dead crystal in ~44,000 hours (5 years)
- Synchrotron - crystals at 77K (close enough to 100K)
  - Brookhaven $\sim 0.5 \times 10^{10}$ photons s^{-1} mm^{-2}
    - Dead crystal in ~ 1.5 days
  - Stanford $\sim 1.2 \times 10^{11}$ photons s^{-1} mm^{-2}
    - Dead crystal in ~ 1.5 hours
  - APS $\sim 1.3 \times 10^{13}$ photons s^{-1} mm^{-2}
    - Dead crystal in ~ 4 seconds

Experimental

- Xylsoe isomerase grown in 3% isopropanol, 20% ethylene glycol, 50 mM $MgCl_2$ HEPES pH 7.0
- Ethylene glycol is a free radical scavanger and potentially useful for mitigating radiation damage as well as acting as a cryoprotectant.
- The crystal size was approximately 200 x 150 x 100 mm.

- Data was collected at beamline 11-1 of the Stanford Synchrotron Radiation Laboratory (SSRL) using an ADSC Quantum 310 detector
- An initial image was collected with l of 0.954 Å, crystal to detector distance of 150 mm, phi oscillation of 0.5º, and exposure time of 2 s.

Experimental contd.

- The data were indexed and a strategy for optimum data collection calculated using Mosflm (Leslie, 1992).
- The dose was normalized to time at this point.
- Following this the wavelength was changed to 0.855 Å and the beam optimized.
- A high-resolution swathe of reciprocal space was then collected with a total of 20 images, 30s equivalent dose exposure, crystal to detector distance of 100 mm and phi oscillation of 0.5º.
- The wavelength was than changed to 0.954 Å and again optimized.
- A complete data set of 180 images, 0.5º oscillation, 2s equivalent dose, and crystal to detector distance was then collected.
- Data collection continued alternating with experimentally identical high-resolution swathes and complete data sets to produce a total of 8 swathes and 7 complete data sets.
- Dose mode was used throughout to maintain a constant X-ray exposure in each case.

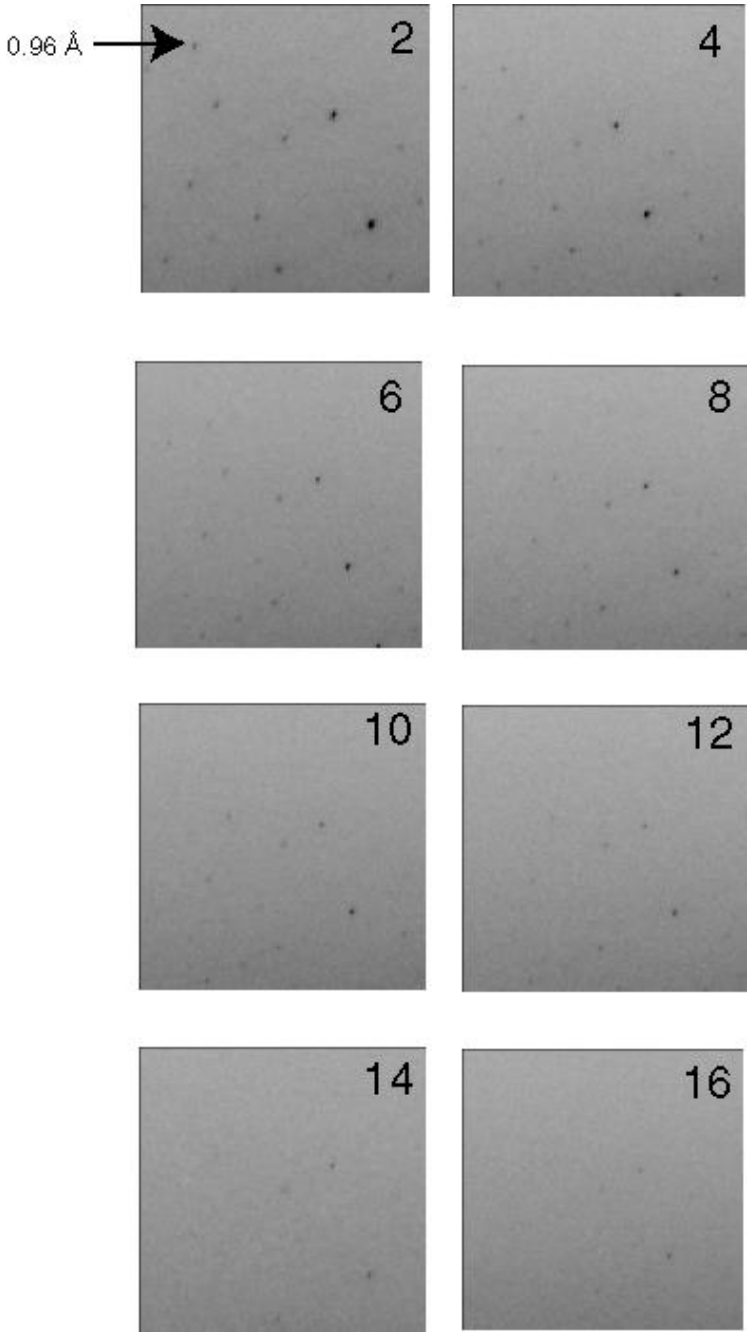
Experimental contd.

- The resulting data were indexed, integrated and reduced using Denzo and Scalepack (Otwinowski and Minor, 1997).
- The B_{factor} was calculated using the program Truncate in the CCP4 suite (Collaborative Computational Project, 1994).
- Normal probability plots (Abrahams and Keve, 1971) show whether data from two crystals are identical or differ systematically and provide information about individual pairs of measurements in addition to the overall agreement.
- Howell and Smith (Howell and Smith, 1992) made use of this technique to identify heavy atom derivatives.
- In this case we used the same technique, through the CCP4 program Scaleit, to look for differences that were manifest in structural changes rather than simple radiation decay

The Numbers

High resolution partial data set (0.9 Å)								
Data set	2	4	6	8	10	12	14	16
R_{factor}	6.7(45.8)	6.7(54.7)	6.9(57.5)	7.2(59.4)	7.5(85.2)	8.0(68.7)	8.0(73.5)	8.0(-)
I/σ(I)	8.9(1.6)	8.5(1.2)	8.6(1.0)	8.3(0.8)	8.3(0.7)	8.0(0.6)	7.8(0.6)	7.7(0.5)
Completeness (%)	24.8(24.8)	24.8(23.2)	24.5(19.6)	24.1(15.3)	23.6(10.9)	23.0(7.0)	22.2(3.1)	21.7(1.4)
Redundancy	1.4(1.4)	1.4(1.3)	1.4(1.2)	1.3(1.2)	1.3(1.1)	1.3(1.1)	1.3(1.0)	1.3(1.0)
Mosaicity (º)	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.16
B_{factor}	6.04	6.35	6.70	6.85	7.25	7.54	7.85	8.13
Medium resolution complete data set (1.2 Å)								
Data set	3	5	7	9	11	13	15	
R_{factor}	7.5(22.5)	7.5(24.7)	7.7(27.3)	7.6(30.1)	7.9(33.4)	7.9(37.3)	7.8(41.7)	
I/σ(I)	16.8(5.0)	16.6(4.7)	16.4(4.3)	16.6(3.9)	16.1(3.3)	15.4(2.8)	15.3(2.4)	
Completeness (%)	99.7(99.3)	99.7(99.4)	99.7(98.9)	99.7(99.1)	99.7(98.4)	99.6(96.8)	99.4(93.7)	
Redundancy	3.6(3.2)	3.6(3.3)	3.5(3.2)	3.5(3.1)	3.5(2.8)	3.5(3.0)	3.5(2.8)	
Mosaicity (º)	0.14	0.14	0.14	0.14	0.14	0.14	0.14	
B_{factor}	8.77	8.83	9.07	9.61	9.83	10.31	10.86	

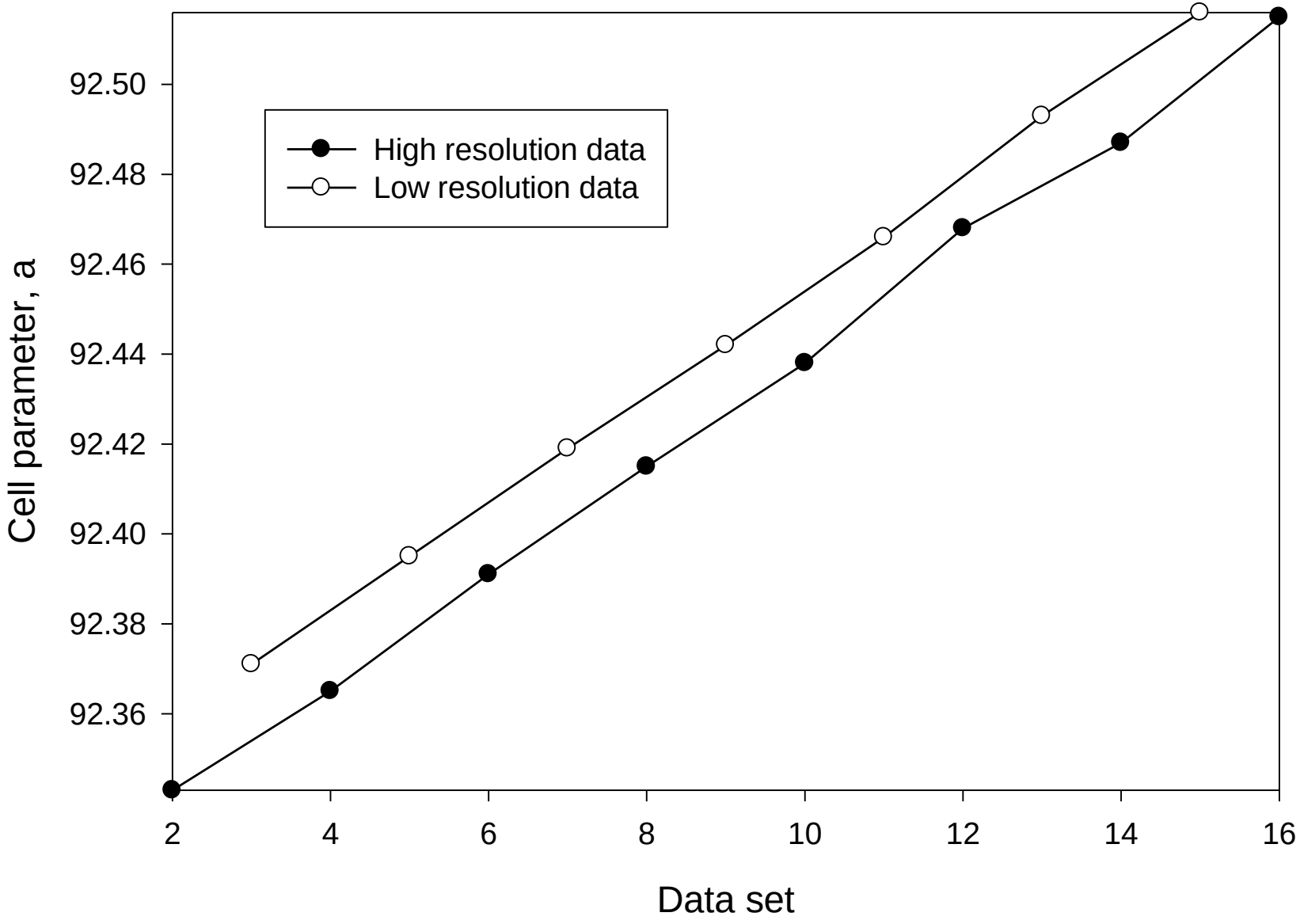
With each data set R_{factor} increases, signal-to-noise, completeness, and redundancy decreases. The mosaicity is unchanged, we are just seeing the beam contributions. The B_{factor} increases.

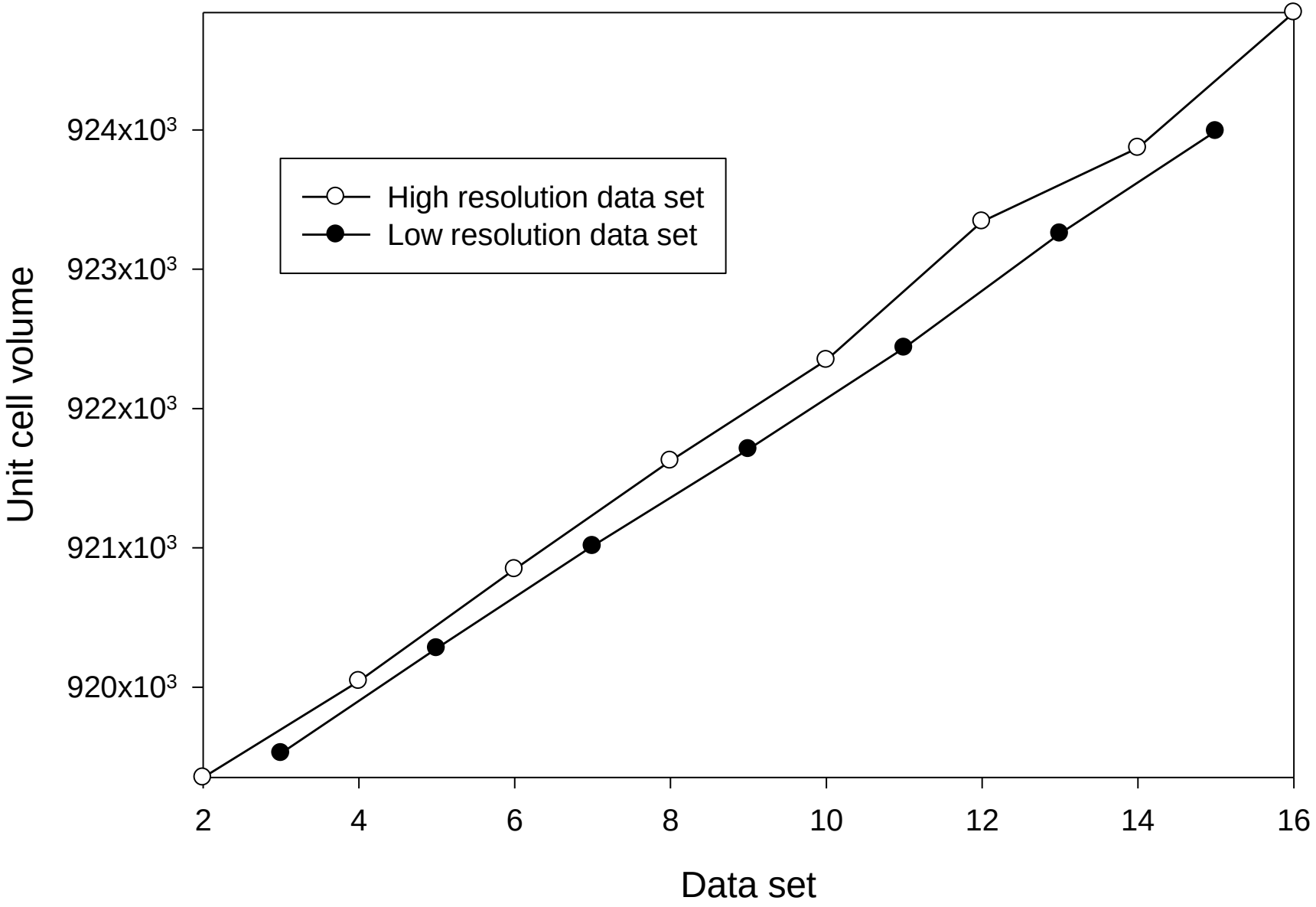


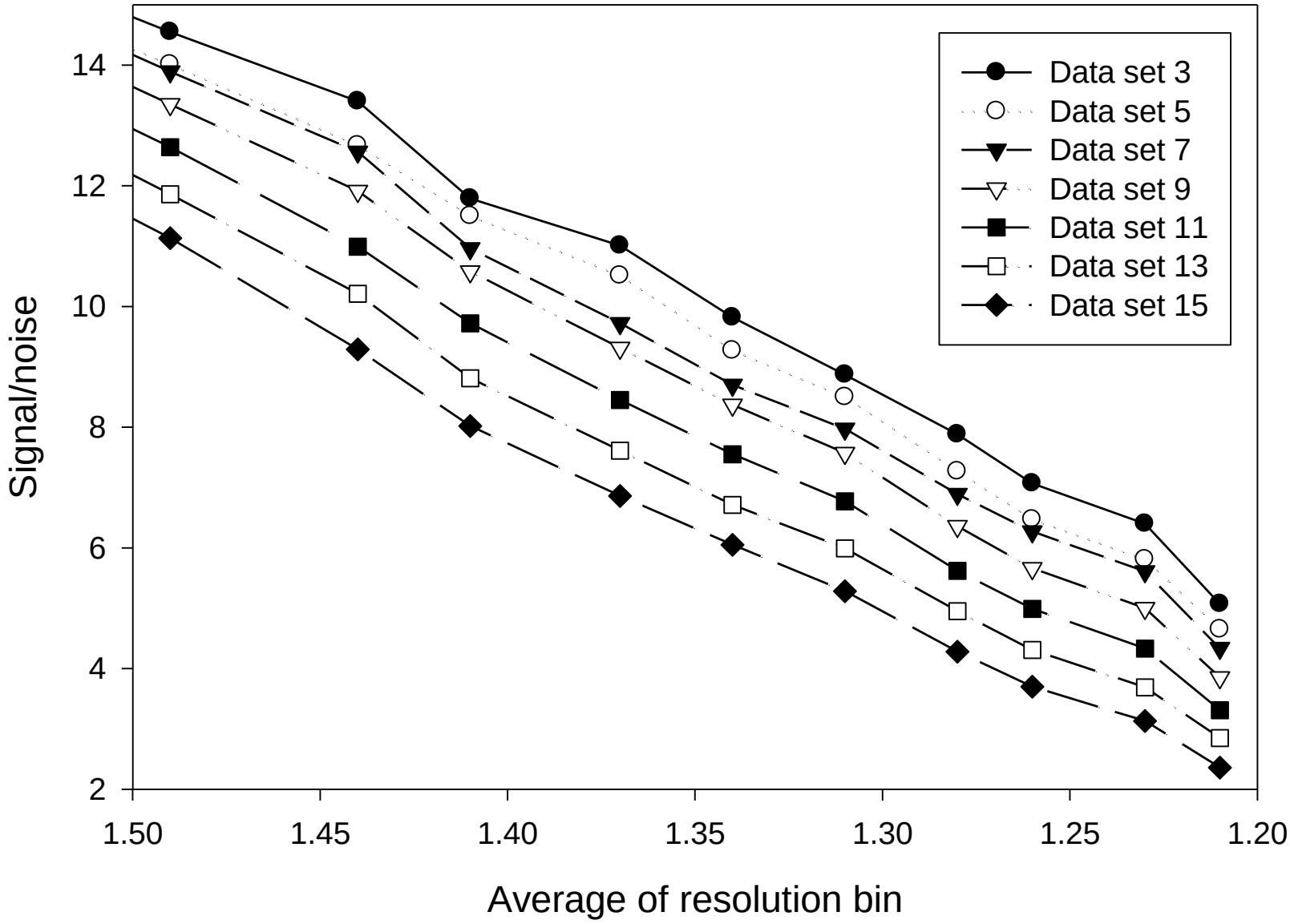
The Images

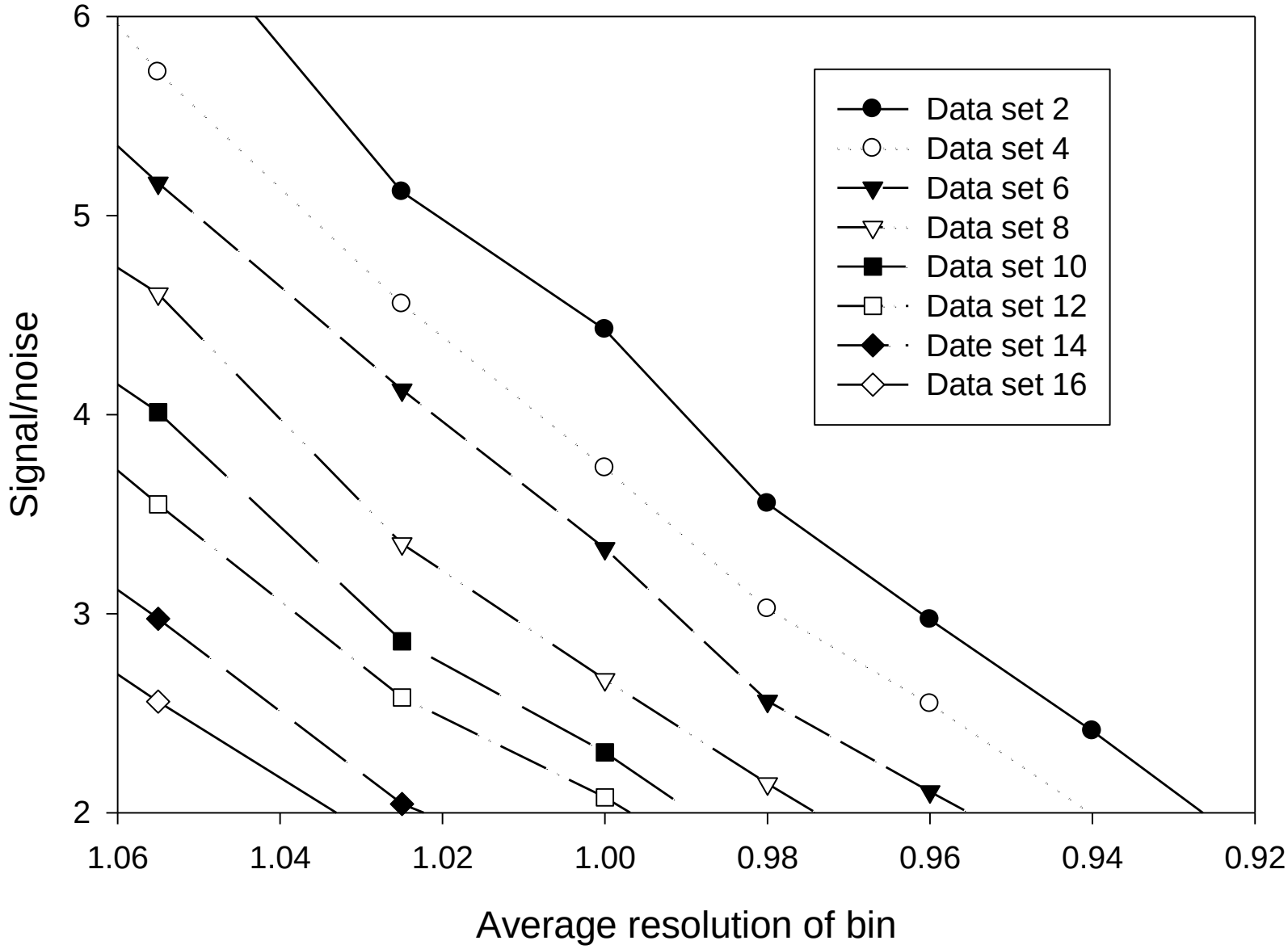
Same portion of high resolution data showing gradual decay of reflections.

Note that the background radiation remains constant


92.50
92.48
92.46
92.44
92.42
92.40
92.38
92.36
Cell parameter, a
High resolution data
Low resolution data
2
4
6
8
10
12
14
16
Data set


924x10³
923x10³
922x10³
921x10³
920x10³
Unit cell volume
High resolution data set
Low resolution data set
2
4
6
8
10
12
14
16
Data set

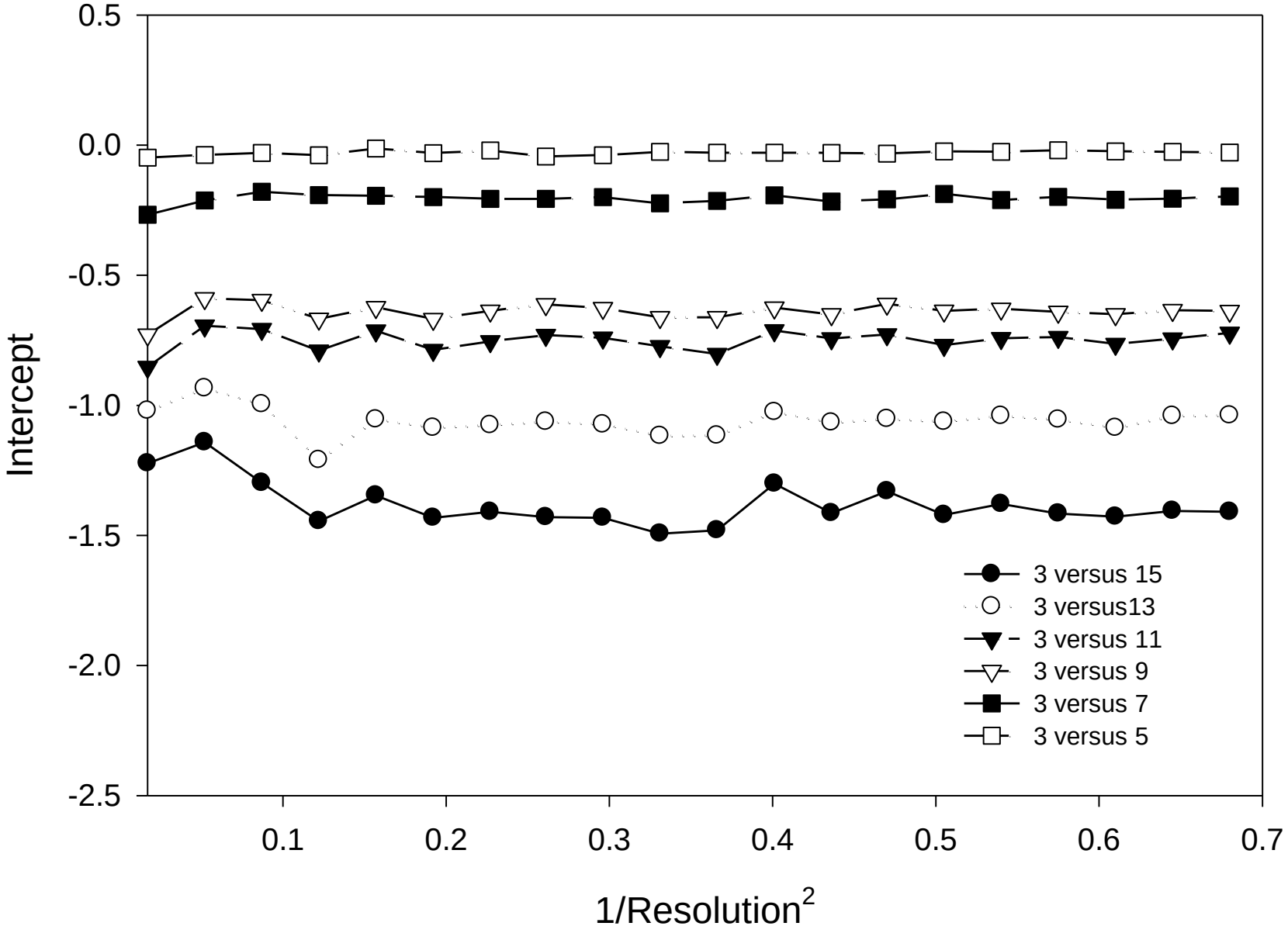

Signal/noise
14
12
10
8
6
4
2
1.50
1.45
1.40
1.35
1.30
1.25
1.20
Average of resolution bin
Data set 3
Data set 5
Data set 7
Data set 9
Data set 11
Data set 13
Data set 15

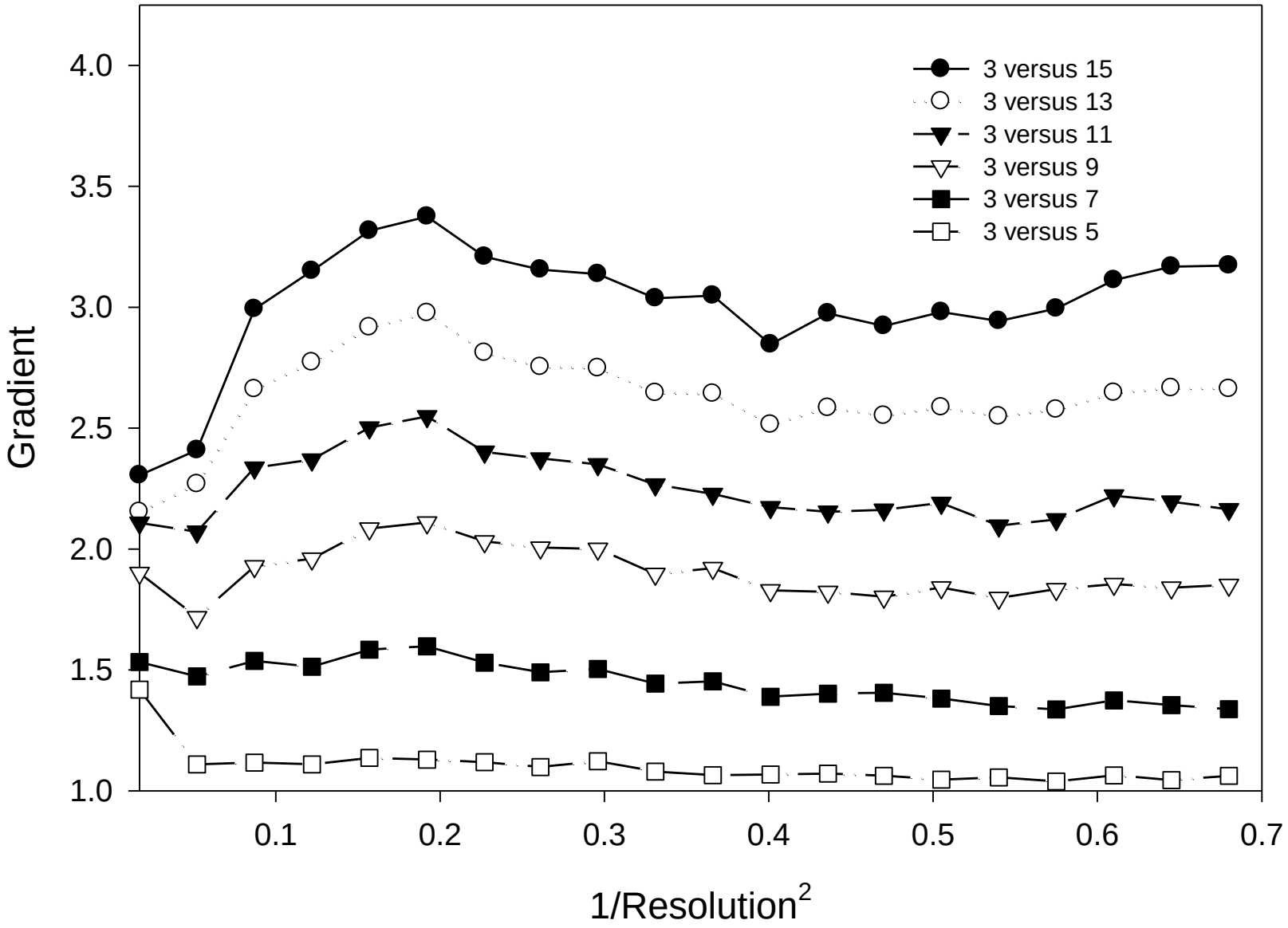

Signal/noise
6
5
4
3
2
1.06
1.04
1.02
1.00
0.98
0.96
0.94
0.92
Average resolution of bin
Data set 2
Data set 4
Data set 6
Data set 8
Data set 10
Data set 12
Date set 14
Data set 16

Structural consequences

- Electron spin resonance shows electrons mobile at 77K
- Specific structural damage
  - Disulphide bridges broken
  - Decarboxylation of glutamate and aspartate residues
  - Tyrosine residues lose their hydroxyl group
  - Methionines: carbon sulphur bond cleaved
- Incomplete data
- Specific structural damage
- Wrong biological information
- Failure of MAD methods

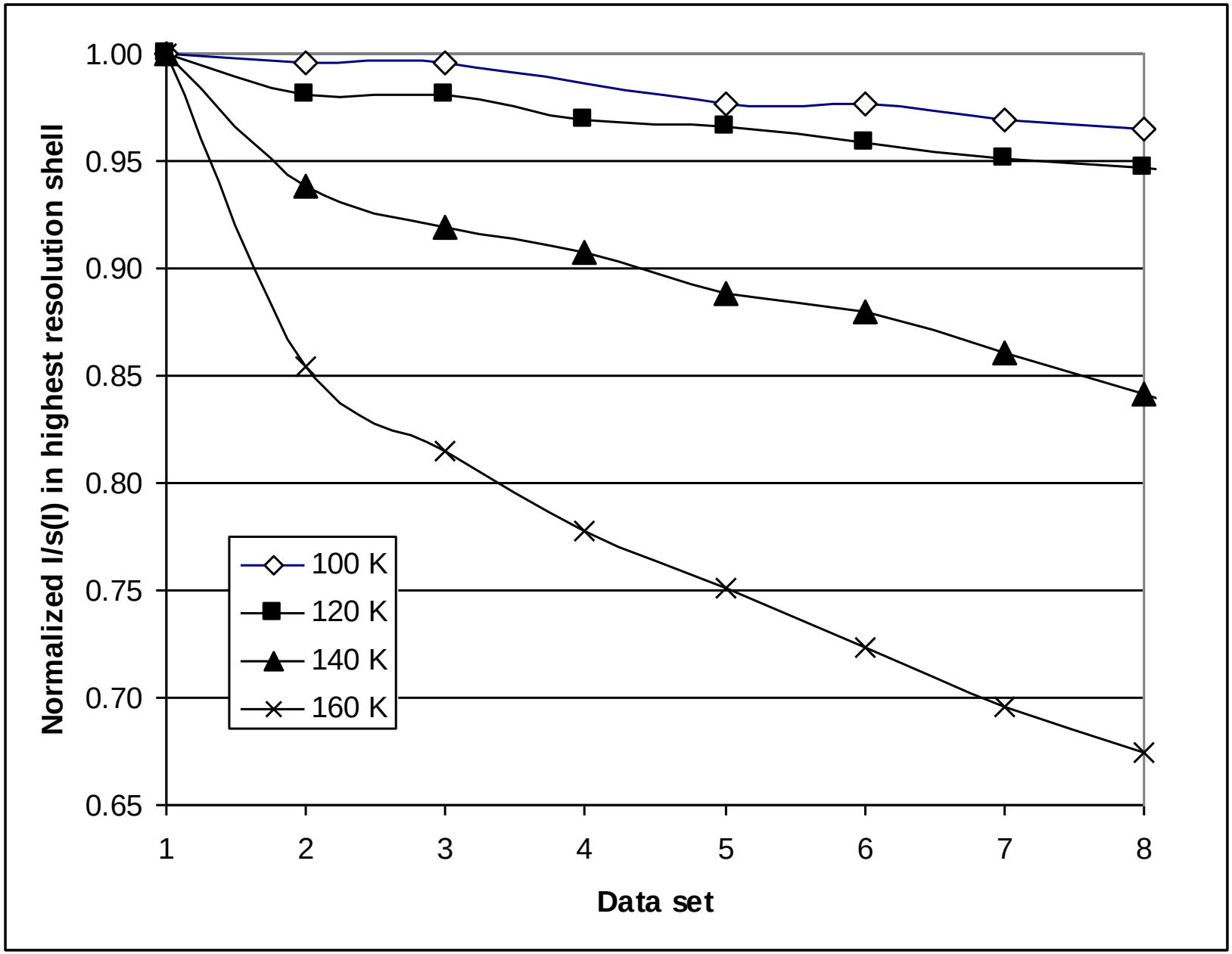
Do we have structural changes in the data?


0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Intercept
0.1
0.2
0.3
0.4
0.5
0.6
0.7
1/Resolution2
3 versus 15
3 versus13
3 versus 11
3 versus 9
3 versus 7
3 versus 5


Gradient
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
1/Resolution^2
3 versus 15
3 versus 13
3 versus 11
3 versus 9
3 versus 7
3 versus 5

What happens if we look at different temperatures?

- Similar experiment
  - Repetitive identical sets of data
  - 4 crystals of similar volume
  - Each crystal collected initially at 100K for baseline data point
  - One crystal collected at 100K
  - The other 3 at 120, 140 and 160K


1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
Normalized I/s(I) in highest resolution shell
1
2
3
4
5
6
7
8
Data set
100 K
120 K
140 K
160 K

Status of research to date

- Clear metric in terms of cell parameter increase
- Similarly linear decrease in signal-to-noise
- Structural effects are present in the data.
- Structural refinement on each data set is beginning.
- Maintaining as low a temperature as possible is important.

Where is it heading?

High resolution structural information on radiation damage process.
Current published studies at about 2 Å

We know radiation damage occurs but what is actually happening?

Is gas CO_2, CO, H_2, O_2 …. A combination or something else. Under active investigation by a number of groups.

Can we reduce or prevent radiation damage?

- Do we need to?
- Free radical scavangers
- Lower temperature (helium)
- Large crystals, translation
- Attenuate source
- Possibilities of different wavelengths
- Neutron sources

Can we make use of the damage?

- Radiation Induced Phasing (RIP) makes us of the selective damage to disulphide bonds for phasing.
- The damage is caused by intense synchrotron radiation on cryocooled samples

- Can we do the same in house on room temperature crystals, i.e. deliberately induce radiation damage and use the damaged data set and undamaged data set for RIP?
- Can we come up with a better acronym?

Acknowledgements

- SSRL for beam time
- Mark van der Woerd, Daren Feree, NASA MSFC
- Funding from NASA
- Elspeth Garman, Oxford University