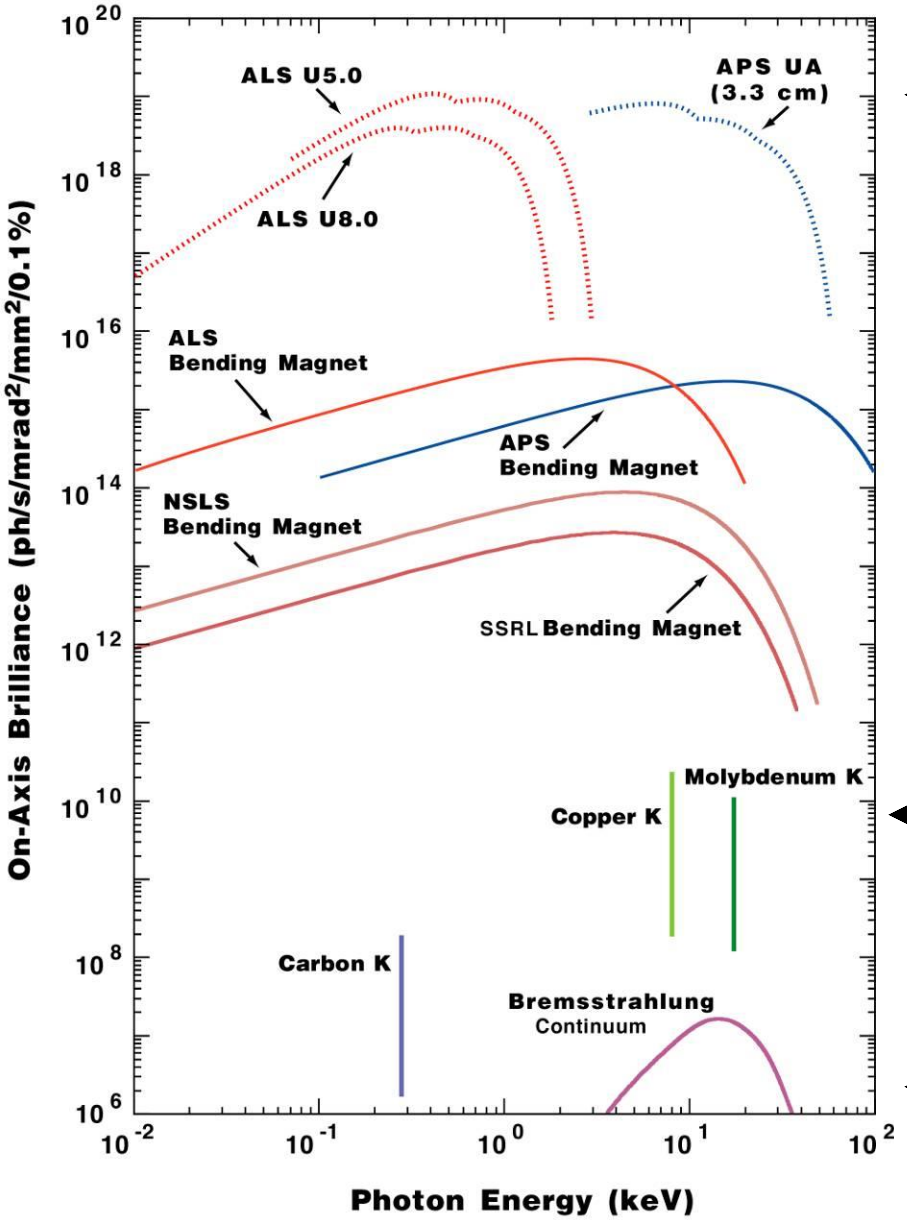
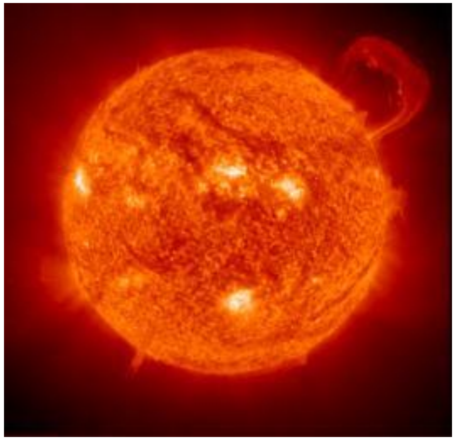
Eddie Snell

HWI

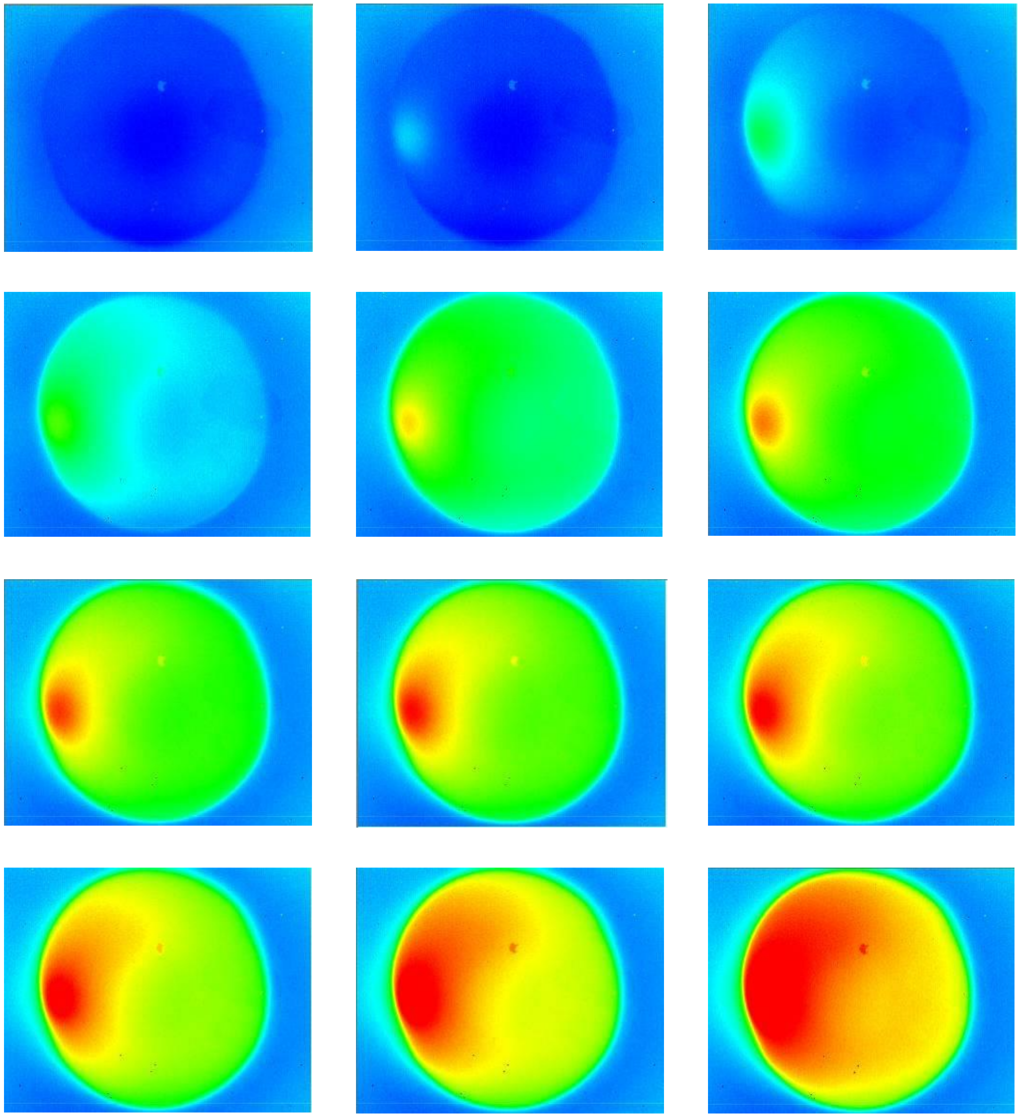
Heat and Radiation effects at Synchrotrons



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



Heat Effects - 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!

However, when cryocooling is present the temperature rise is only on the order of 4K

The phase transition for ice transition is ~140K therefore beam heating is not a problem.

Radiation Damage in Macromolecular Crystals

- 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?

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.

X-ray radiation effects on water

Ionizing radiation can remove an electron from water: $H_2O^+ + H_2O \rightarrow H_3O^+ + OH$ and the ejected electron: $e^- + H_2O \rightarrow OH^- + OH$

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

$H + OH \longrightarrow H_2O$ $\quad$ $H + H \longrightarrow H_2$ $\quad$ $OH + OH \longrightarrow H_2O_2$

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.

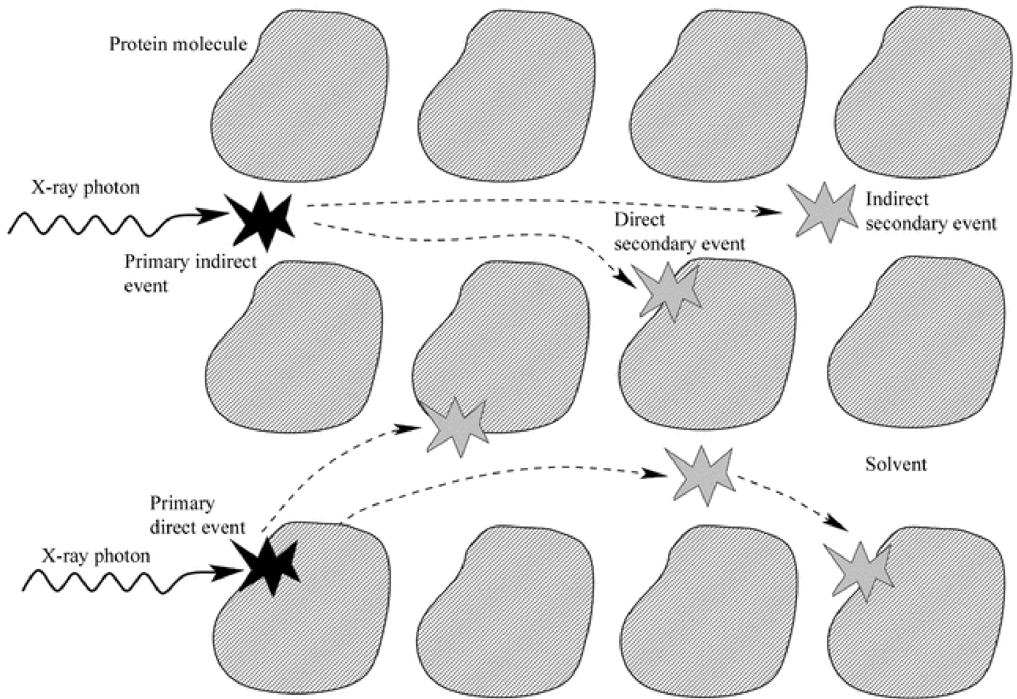
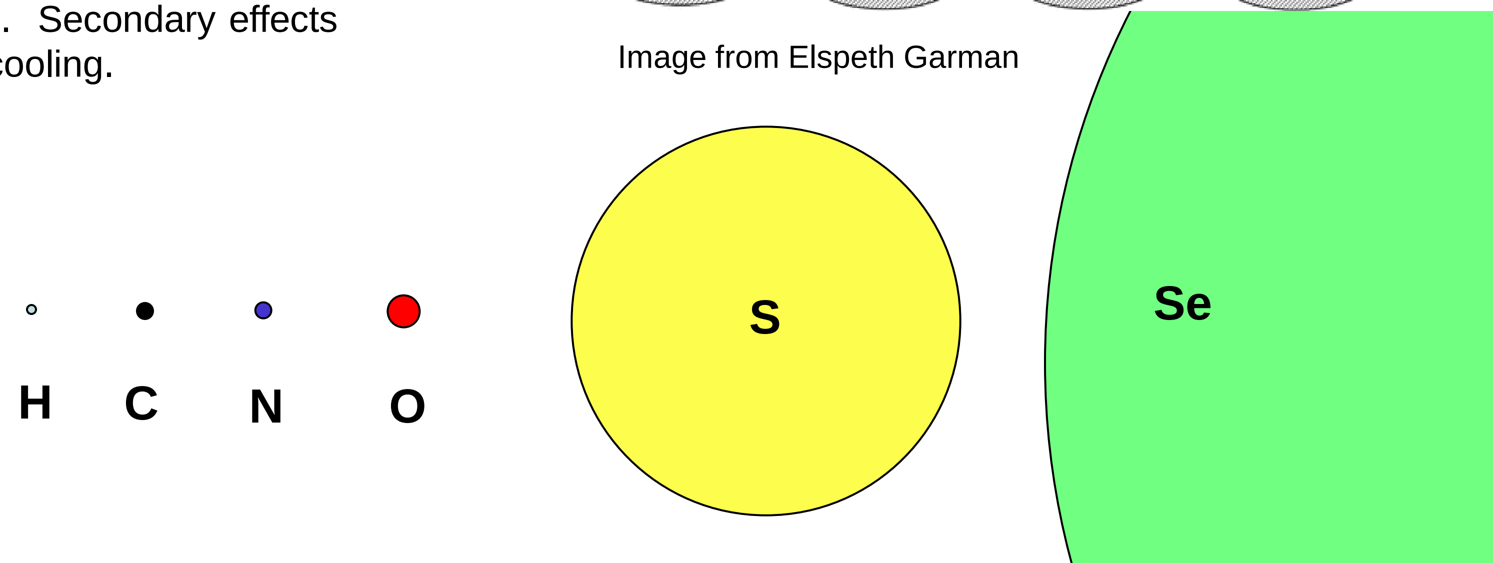


Image from Elspeth Garman

Effect on Other atoms



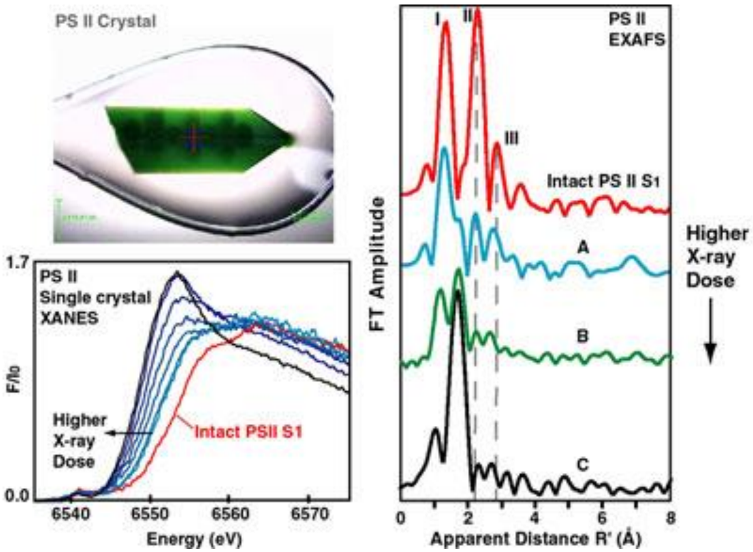
Henderson Limit

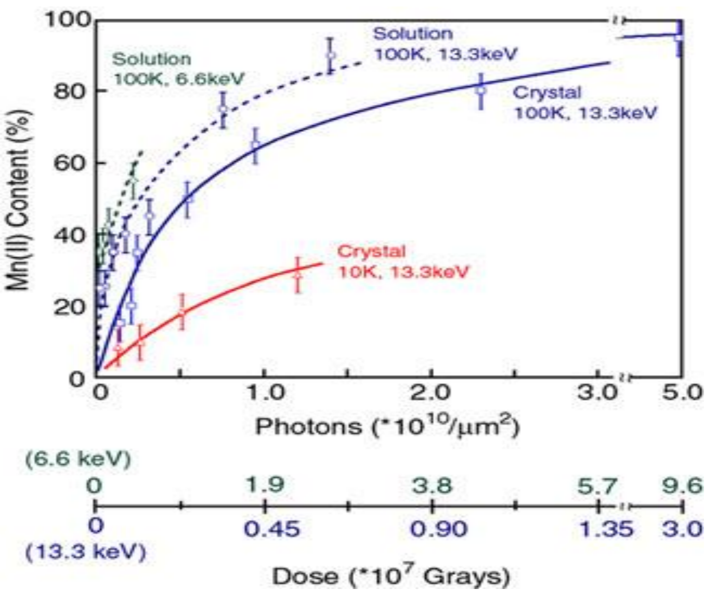
- Radiation damage by electrons and X-rays are comparable.
- Electron diffraction patterns fade to ½ their original intensity after 1 electron $Å^{-1}$ at room temperature or 5 electron $Å^{-1}$ at 77K.
- The amount of energy absorbed per unit weight is expressed in units of gray (Gy). One gray dose is equivalent to one joule radiation energy absorbed per kilogram. One gray is equivalent to 100 rads.
- 5 electrons $Å^{-1}$ is approx 5×10^{7} Gy.
- The depth dose curve (maximum dose at ~100 μm) reduces the energy deposition so the effective energy causing the damage is conservatively 2×10^{7} Gy.
- X-rays of 1.5 Å give 12×10^{-16} Gy per photon m^{-2}.
- The X-ray flux giving rise to 2×10^{7} Grays is 1.6×10^{16} photons mm^{-2}

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

What does it mean practically?

- Synchrotron - crystals at 77K (close enough to 100K)
  - Brookhaven ~0.5×10^{10} photons s^{-1} mm^{-2}
    - Dead crystal in ~ 1.5 days
  - Stanford ~1.2×10^{11} photons s^{-1} mm^{-2}
    - Dead crystal in ~ 1.5 hours
  - APS ~1.3×10^{13} photons s^{-1} mm^{-2}
    - Dead crystal in ~ 4 seconds





Case study - Photosystem II

Yano, J et al *Proc. Natl. Acad. Sci. USA* **2005**, *102* 12047-12052

As the X-ray dose increases, Mn normally present as Mn4(III2,IV2) is reduced to Mn(II) as seen by the changes in XANES spectra (left). The changes in the corresponding EXAFS spectra (right) show that the three Fourier peaks characteristic of Mn-bridging-oxo, Mn-terminal, and Mn-Mn/Ca interactions (dashed vertical line) are replaced by one Fourier peak characteristic of a Mn(II) environment.

Increasing Mn(II) content due to radiation damage. (Solid blue line) Mn(II) content in the crystals as a function of X-ray irradiation at 13.3 keV (0.933 Å) at 100 K - similar to those during x-ray diffraction data collection. At 66% of the dose (2.3x1010 photons/μm2) compared to the representative average dose of (3.5x1010 photons/μm2) used for crystallography, the crystals contain ~80% Mn(II). (Dashed blue line) The damage profile for solution samples is similar to that seen for crystals. (Dashed green line) The generation of Mn(II) is considerably greater when the x-ray irradiation is at 6.6 keV (1.89 Å) which is the energy at which the anomalous diffraction measurements were conducted. (Solid blue line)

Experimental – What is happening on the atomic scale

- 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 sizes were approximately 200 x 150 x 100 μm.
- Data was collected at beamline 11-1 of the Stanford Synchrotron Radiation Laboratory (SSRL) using an ADSC Quantum 310 detector
- Two crystals were used – one for a high-resolution base-line data set (low, medium and high resolution swathes in that order).
- 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. The dose was normalized to time at this point.
- The data were indexed and a strategy for optimum data collection calculated using Mosflm (Leslie, 1992).
- 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.

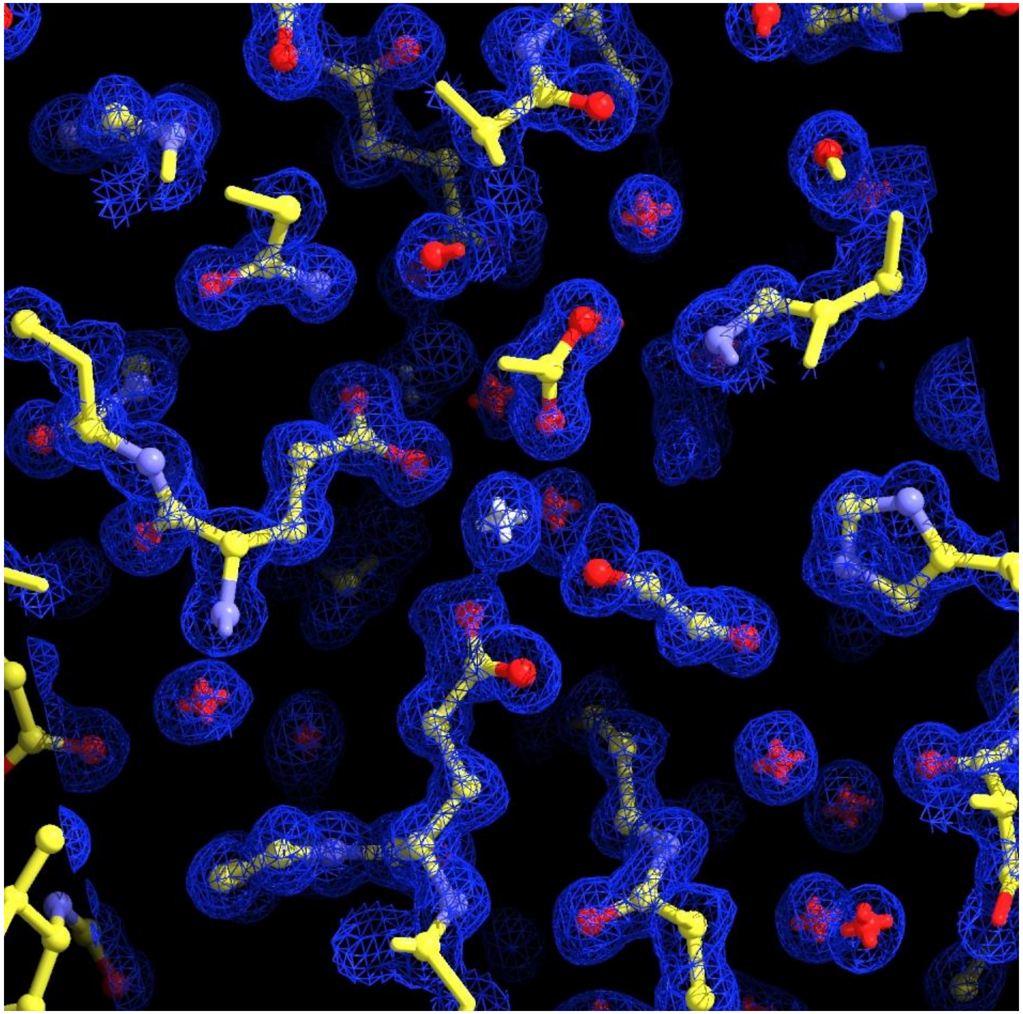
- The resulting data were indexed, integrated and reduced using Denzo and Scalepack (Otwinowski and Minor, 1997).
- The B_{factor} was calculated using the program Truncate in the CCP4 suite (Collaborative Computational Project, 1994).
- Normal probability plots (Abrahams and Keve, 1971) show whether 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

Current work underway

Structural refinement of each data set

- Arp/Warp to remove initial model bias
- Coot to fit model to density and model in cryoprotectant
- Refmac for further refinement with Warp used for water positions
- Procheck and Coot internal routines used to check result
- Iteration Coot, Refmac, Procheck

Baseline High-Resolution Model



- I222 space group, a=92.69, b=97.87, c=102.24
- 40-0.87A resolution
- 3,989,654 reflections
- 376,419 unique
- 10.6 (9.3) multiplicity
- I/s(I)=19.61 (2.03)
- Rmerge 7.5% (82.1%)
- Rrim 7.9% (82.8%)
- Rpim 2.4% (26.1%)
- Average chi 0.954
- Completeness 99.5 (82.9)

- Structural refinement
- 3 cycles to date
- R factor 17.86
- Free R factor 19.25
- Many multiple conformations

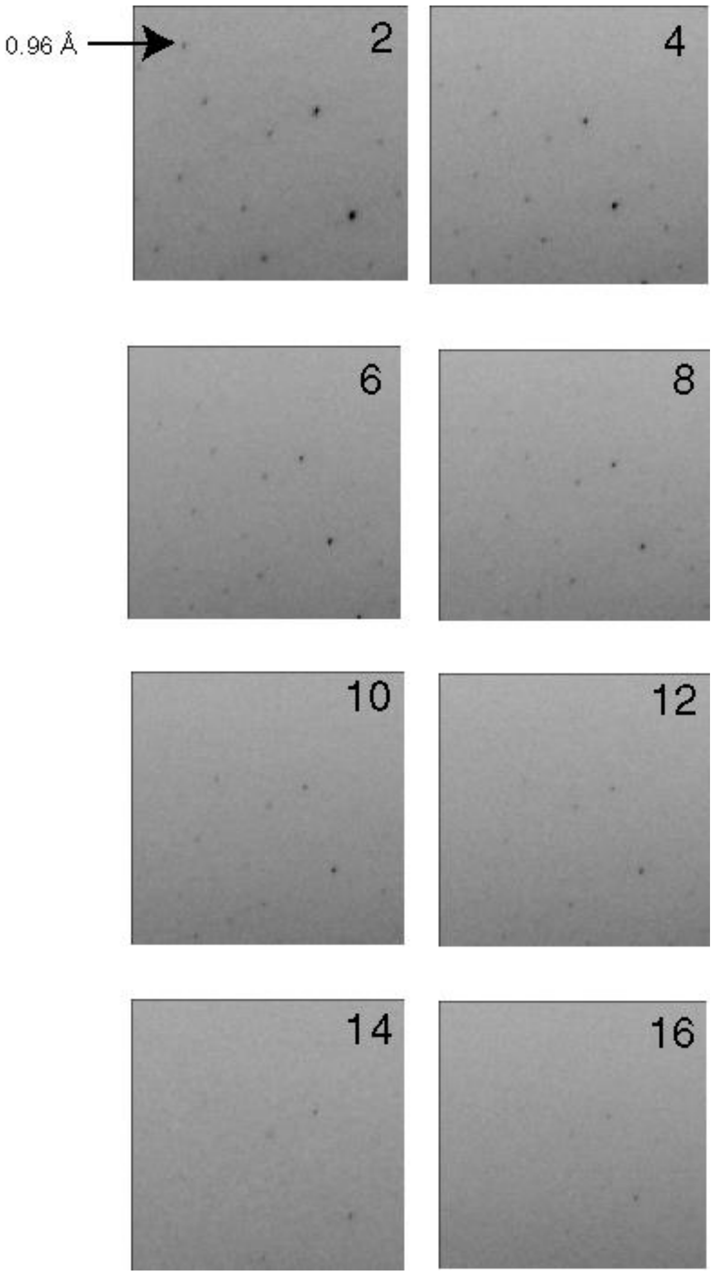
2Fo-Fc map contoured at 2 sigma

The Numbers – Radiation Damage Datasets

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(-)
$I/\sigma(I)$	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)	
$I/\sigma(I)$	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	

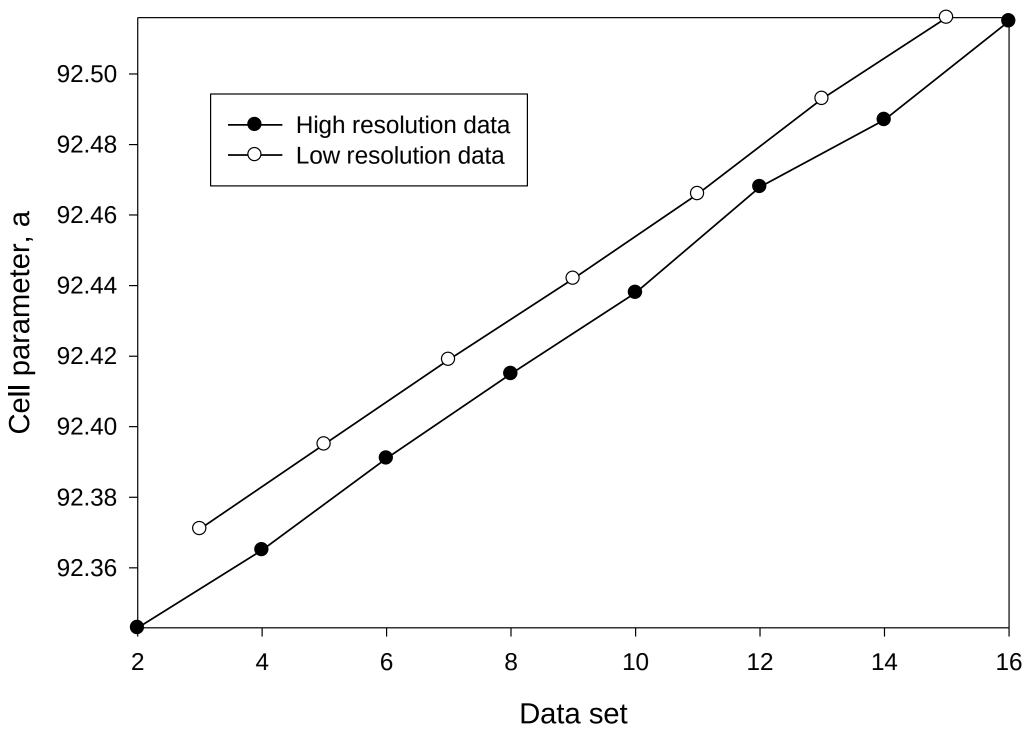
With each data set R_{factor} increases, signal-to-noise, completeness, and redundancy decreases. The mosaicity is unchanged, we are just seeing the beam contributions. The B_{factor} increases.

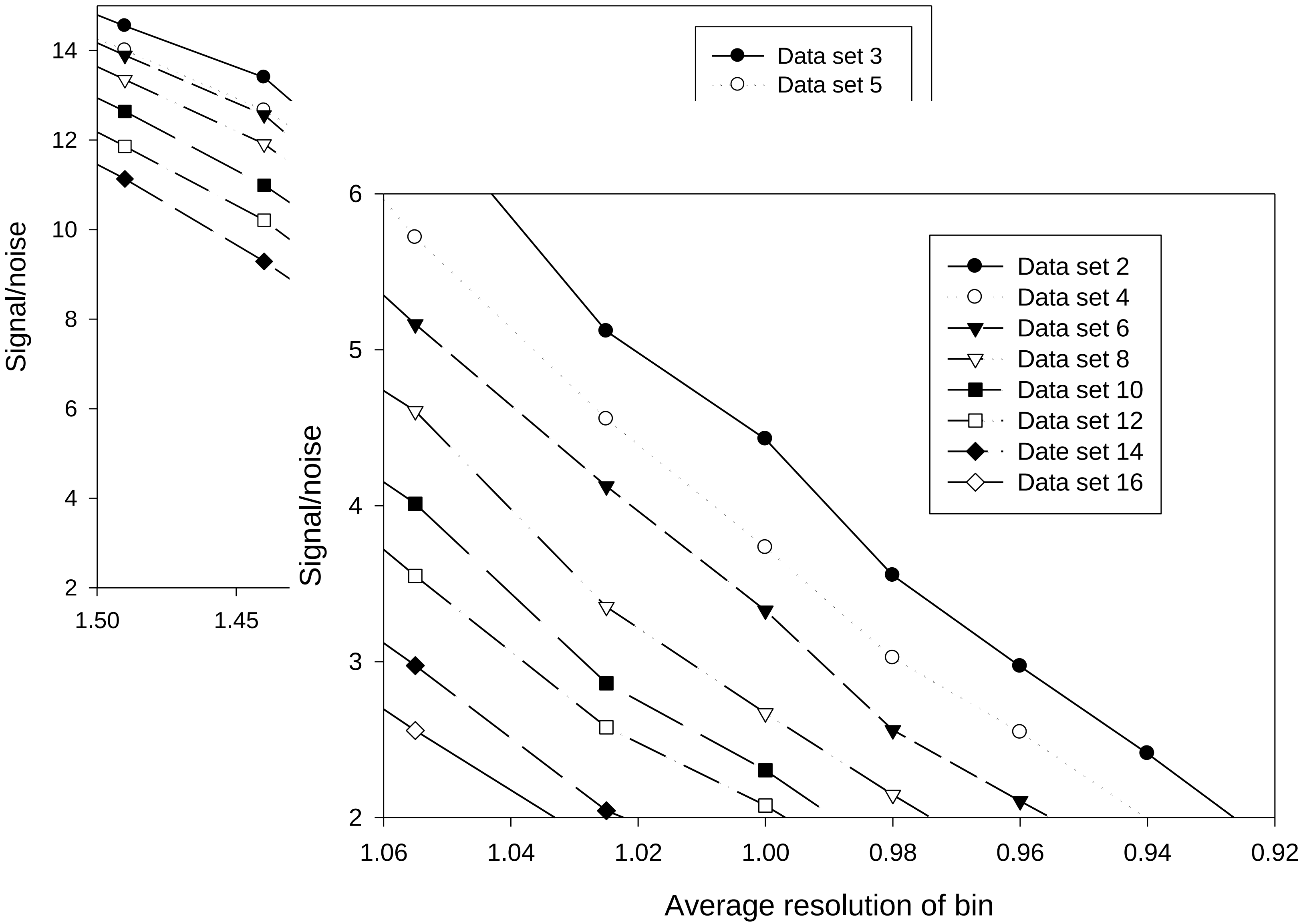
The Images



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

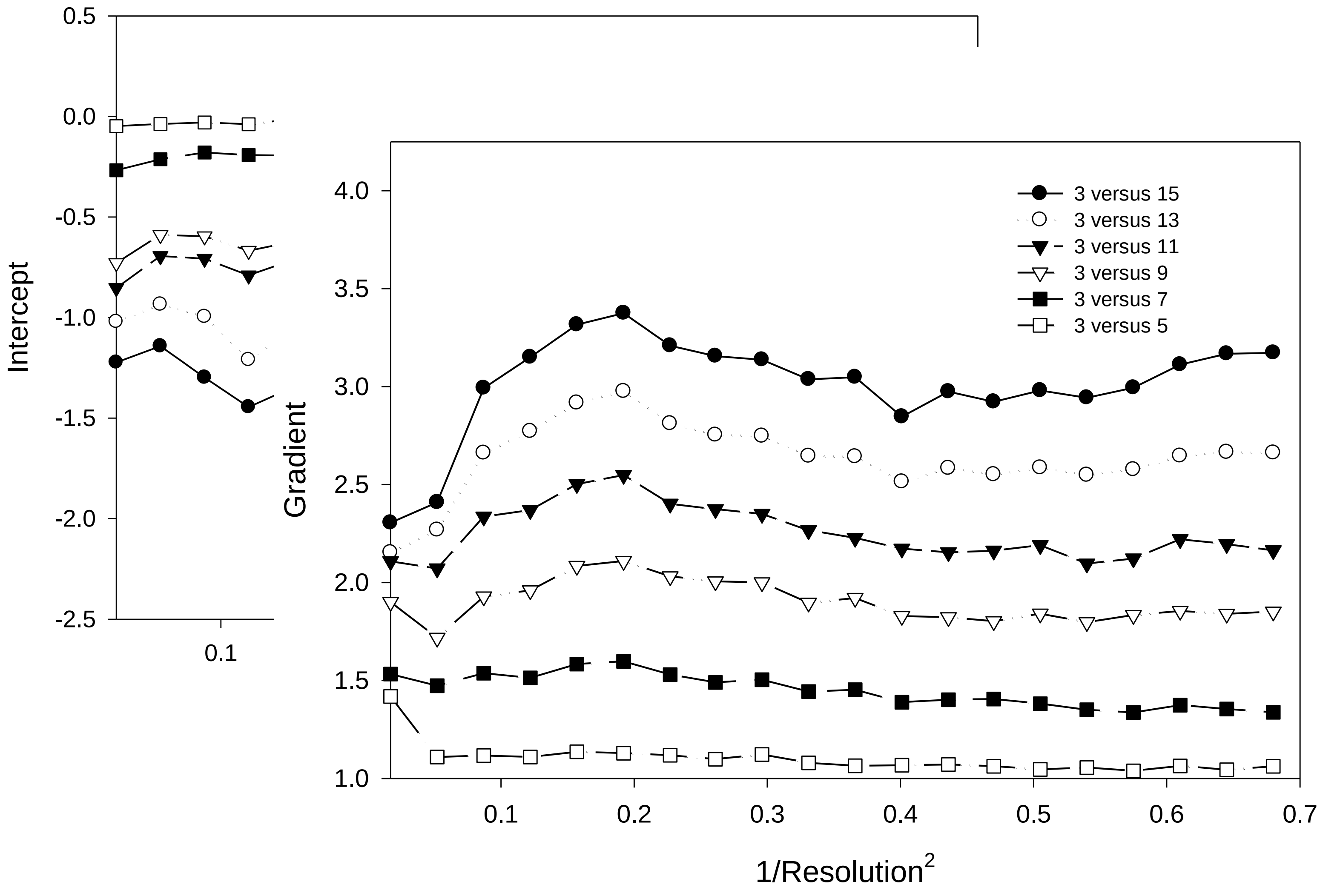
Note that the background radiation remains constant





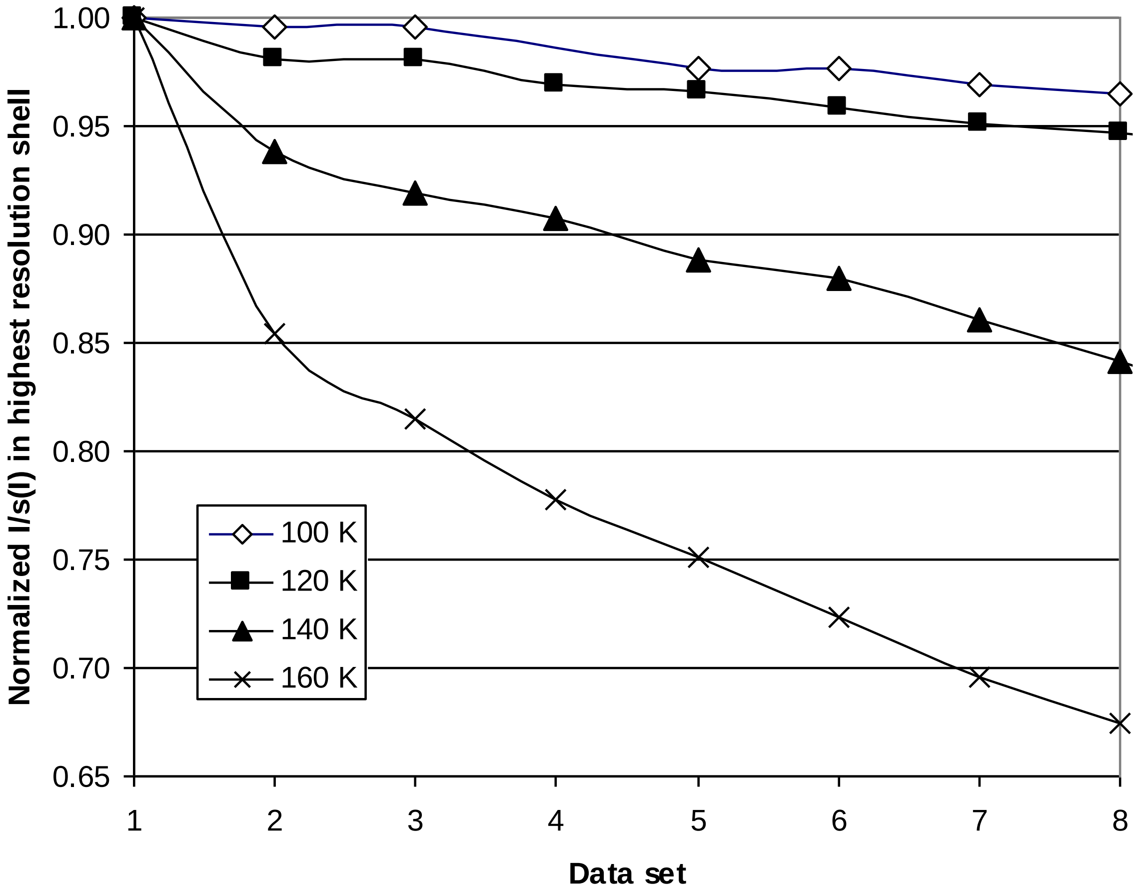
Data set 3
Data set 5
Signal/noise
14
12
10
8
6
4
2
1.50
1.45
Data set 2
Data set 4
Data set 6
Data set 8
Data set 10
Data set 12
Date set 14
Data set 16
6
5
4
3
2
1.06
1.04
1.02
1.00
0.98
0.96
0.94
0.92
Average resolution of bin

Are there structural consequences?



Yes, but need the structural refinement before knowing what they are.

What effect does temperature have on radiation damage?



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

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.

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.
- Structural refinement started.

Where is it heading?

- High resolution structural information on radiation damage process - current published studies at about 2 Å
- Data combined with neutron data for charge density studies.
- Complete knowledge about the enzymatic mechanism of xylsoe isomerase.

Questions to ask for the future

- Can we reduce, prevent or make use of the damage? (translate crystals, attenuate beam, scavangers, cryoprotectants)
- What is the process?
- What warning signs do we need to look for to differentiate from mechanism or radiation damage in our structure?

Acknowledgements

- Stanford Synchrotron Radiation Laboratory and the Advanced Photon Source for beam time
- Mark van der Woerd, Daren Feree, NASA Marshall Space Flight Center
- Gerd Rosenbaum, Advanced Photon Source
- Funding from NASA
- Elspeth Garman, Oxford University
- Bob Blessing, Hauptman Woodward Medical Research Institute