Argonne NATIONAL LABORATORY

X-Ray Damage to Biological Crystalline Samples

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International Workshops on X-Ray Damage to Biological Crystalline Samples

First Workshop (RD1) 1999 at ESRF

Second Workshop (RD2) 2001 at APS

http://www.aps.anl.gov/News/Conferences/2001/damage/home.html

8 papers in Journal of Synchrotron Radiation (2002) **9**, 327 - 382

Third Workshop (RD3) 2003 at ESRF

9 papers in Journal of Synchrotron Radiation (2005) **12**, 257 - 329

Fourth Workshop (RD4) 2006 at SPring8

http://www.spring8.or.jp/en/users/meeting/rd4

several presentations will be published

Organizers:
Elspeth Garman, Collin Nave, Gerd Rosenbaum, Raimond Ravelli, Seam McSweeney

Radiation Damage - An Unavoidable Byproduct Of Crystallographic Data Acquisition

- Radiation damage is proportional to dose on sample.

  dose = absorbed energy / mass (unit: 1 Gy = 1 J/kg)

  dose = μ/ρ I t ε_{phot} I = flux density, t = exp. time

- Number of photons in diffraction peak

  $N_{phot} \sim I\ t\ V\ \lambda^2$ V = sample volume

- Dose on sample / number of photons in diffraction peak

  dose / $N_{phot} \sim 1/V$

  Almost all deposited energy is by photoelectric absorption.

  $(\mu/\rho)_{photo} \sim \lambda^3$ => dose / $N_{phot} \sim \lambda^2/\lambda^2$

- Radiation damage to cryo-cooled samples is **not** a particular property of 3rd gen. sources.

  Lavish use of available flux **is** characteristic for users of 3rd gen. sources: over-exposure

  Detector read-out units should be calibrated in photons to help guide level of exposure.

Primary and Secondary Radiation Damage and Cryo-cooling

Primary radiation damage:

Direct hit of protein by absorbed photon or by ejected photo-electron

Secondary radiation damage:|

Damage of protein by action of non-protein molecules activated by absorbed photon or by photo-electron (mostly hydroxyl radicals)

Data Collection at Room Temperature:

Early observation of radiation damage on home sources.

More than 1 sample per data set needed.

Cryo-cooling of samples:

Radicals immobilized at 100 K; secondary damage stopped;

~50-100-times increase in sample life (dose tolerance); $I_{1/2}$ dose = $4*10^7$ Gy

Cooling to 15 K: improvement not clearly demonstrated; B-factor reduced (mostly)

Radiation Damage Observed at 3rd Generation Sources

Renewed Attention to Radiation Damage at 3rd Generation Sources:

- observed higher radiation damage attributed to high flux density
- no significant dose rate effect observed in carefully designed studies
- photons from 3rd gen. sources are not different from photons from other sources
- high flux allows much higher exposure in available beam time allotment
- concern about beam heating:
  not the cause of increased damage, even at max. flux from APS undulator
  temperature increase <10 K

X-Ray Damage is Not Local

- photo-electrons carry ~95% of energy of absorbed photon (12 keV)
- photo-electrons from 12 keV photons travel ~2.8 μm
- transfer damage energy of 20 eV average per hit
- only 5% of energy (binding energy of C, N, O) of absorbed photon stays within 5 Å

Effects of Radiation Damage

Breaking bonds => loss of occupancy, increased temp. factor
- S-S bonds first, then carboxyl, then other charged residues, then …
- (It's the chemistry, not the local absorption cross-section.)

Changing charge state
- Reduction of metal centers
- Ironically, cryo-protectant among worst promoters.

Increase in unit cell volume and lattice constants

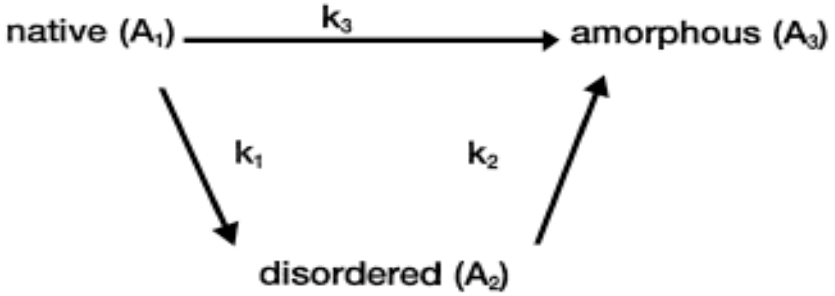
General decrease of intensity of diffraction peaks

Increase in B-factor

Non-Isomorphism
- Increased R_{merge} between initial and later frames
- Problem for SAD/MAD phasing, especially sulfur SAD
- (Byfoet pairs suffered different amount of radiation damage)

Process of Radiation Damage

Blake and Phillips [1] suggest the following model for radiation damage :



The "disordered" unit cells have elevated temperature factors; the amorphous regions do not give Bragg diffraction at all. Hendrickson [2] concluded from data collected from myoglobin at room temperature that k_3=0. Sliz et al. [3] concluded from data collected from several samples at 100 K at much higher exposure that k_3=0.

The process of radiation damage is sequential: A_1 => A_2 => A_3

1. Blake, C.C. & Phillips, D.C. (1962). Effects of X-irradiation on single crystals of myoglobin. In Biological Effects of Ionizing Radiation at the Molecular Level. (Vienna: International Atomic Energy Agency), pp. 183-191.
2. Hendrickson, W.A. (1976). Radiation damage in protein crystallography. J. Mol. Biol. *106*, 889-893.
3. Sliz, P., Harrison, S.C. & Rosenbaum, G. (2003).How does Radiation Damage in Protein Crystals Depend on X-Ray Dose. Structure *11*, 13-19

Damage vs. Dose, Dose Rate
Minimum Sample Size

Studies of damage vs. dose and limiting sample sizes by Teng & Moffat (2000, 2002), Glaser et al. (2000) and Sliz, Harrison, Rosenbaum (2003)

Radiation damage proportional to dose up to a certain limit; then fast collapse of structure (non-linear dose effect, not dose rate effect)

No dose rate effect noticeable for flux densities up to $3*10^{15}$ ph/s/mm^2

T&M and S,H,R conclude damage in cryo-cooled samples is primary damage, i.e. radicals are immobilized.

Limit of crystal volume for full data set:
Glaser et al.: ~ 35μm
Teng&Moffat: ~30 μm
Sliz, Harrison, Rosenbaum: ~15 μm
(different resolutions, different criteria for damage limit.)

Typical Effects of Radiation Damage on Diffraction Intensities, R_{merge}, Unit Cell Volume, B-factor

Fig 1

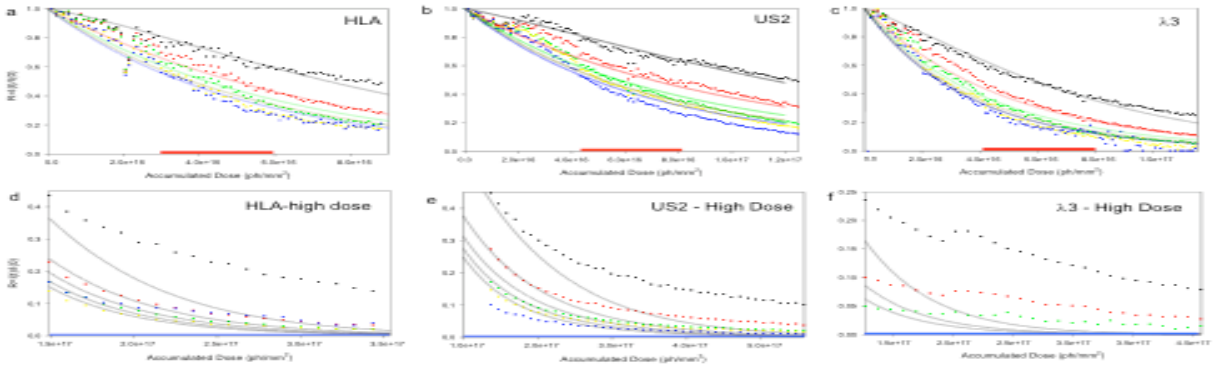


Fig 2

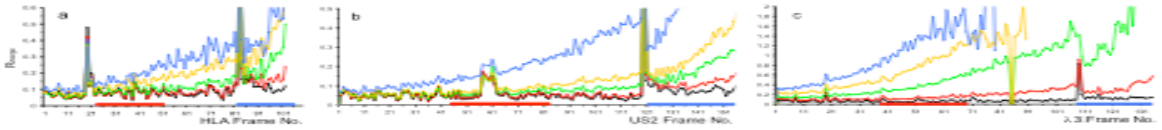
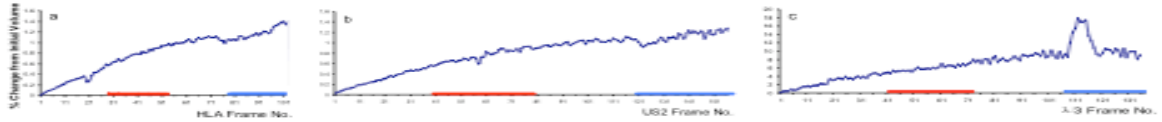


Fig 3



Preventing, mitigating the effects of radiation damage

- **Minimize dose**
  - avoid high Z atoms in buffer
  - calculate dose using program RADDOSE [1]
  - reduce exposure to level necessary for accuracy as determined by photon statistics
  - reduce scatter by
    - minimizing beam on non-crystal volume
    - minimizing path length of primary beam through air
    - clean freeze, no ice
    - avoid high Z atoms in buffer (e.g. arsenic) or other high mol. weight stuff

- Radio-protectants (Garman, others)
  Scavengers: effectiveness not clear; concern about chemistry

1. Murray et al., J.Appl.Cryst. **37**, 513-522
(to get program contact Elspeth Garman elspeth@biop.ox.ac.uk)

Mitigating or make use of the effects of radiation damage

- Correction of diffraction peak intensities for radiation damage (Otwinowsky, others)
  Akin to dead time loss correction. Extrapolate back to zero exposure

- Use non-isomorphism for phasing (Ravelli, others)

- Technology developments that can help reducing dose
  - detectors with low read noise / signal per photon)
  - ratio (read noise / signal per photon) should be <1
  - detectors with large dynamic range
    (max. no. of photons per pixel / read noise in photons)