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

Third Workshop (RD3) 2003 at ESRF

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.
  \[
  \text{dose} = \frac{\text{absorbed energy}}{\text{mass}} \quad \text{(unit: } 1 \text{ Gy} = 1 \text{ J/kg })
  \]
  \[
  \text{dose} = \frac{\mu}{\rho} I t \varepsilon_{\text{phot}} \quad I = \text{flux density, } t = \text{exp. time}
  \]

- Number of photons in diffraction peak
  \[
  N_{\text{phot}} \sim I t V \lambda^2 \quad V = \text{sample volume}
  \]

- Dose on sample / number of photons in diffraction peak
  \[
  \frac{\text{dose}}{N_{\text{phot}}} \sim \frac{1}{V}
  \]
  Almost all deposited energy is by photoelectric absorption.
  \[
  (\frac{\mu}{\rho})_{\text{photo}} \sim \lambda^3 \quad \Rightarrow \quad \frac{\text{dose}}{N_{\text{phot}}} \sim \frac{\lambda^2}{\lambda^2}
  \]

- 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 = $4 \times 10^7$ 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)
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: $A_1 \Rightarrow A_2 \Rightarrow A_3$

**Damage vs. Dose, Dose Rate Minimum Sample Size**


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 $3 \times 10^{15}$ 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.: ~35 µm
- Teng&Moffat: ~30 µm
- Sliz, Harrison, Rosenbaum: ~15 µm

(different resolutions, different criteria for damage limit.)
Typical Effects of Radiation Damage on Diffraction Intensities, $R_{\text{merge}}$, Unit Cell Volume, B-factor
Preventing, mitigating the effects of radiation damage

• Minimize dose
  - avoid high Z atoms in buffer
  - calculate dose using program RADDOS [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

   (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)