Quantitative Radiation Damage Studies

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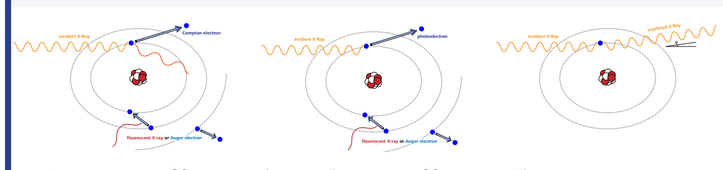
Radiation Damage

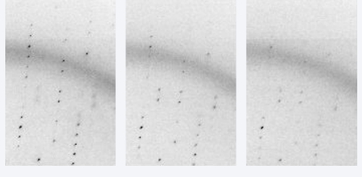
There are three relevant interactions between an X-ray beam and a protein crystal. X-ray crystallography is based on elastic scattering. Interference of scattered X-rays gives rise to a diffraction pattern on the detector.



Compton effect Photoelectric effect Elastic scattering

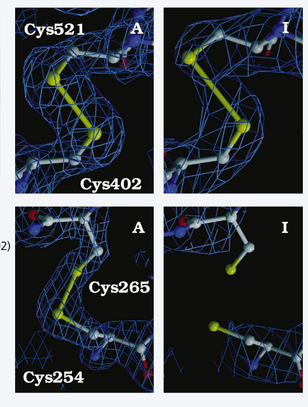
The more prevalent photoelectric and Compton effects cause electrons to be ejected from atoms. These electrons then roam the crystal, further destroying the sample. This causes global and specific radiation damage.

Global damage describes the loss of long range crystal order. The diffraction pattern fades: high resolution information about the protein structure is lost.



Above: Fading diffraction pattern

Right: Disulphide bonds breaking (Weik 2002)



Specific damage describes distinct changes in the protein structure, such as the breaking of disulphide bonds.

Statistical Modelling

The Protein Data Bank currently contains 87,524 deposited structures. With a high redundancy of some proteins and an overall selection bias towards crystallizable protein the PDB is not a representative sample of all proteins.

A non-redundant subset of the PDB has been investigated specifically looking for radiation damage patterns around disulphide bonds.

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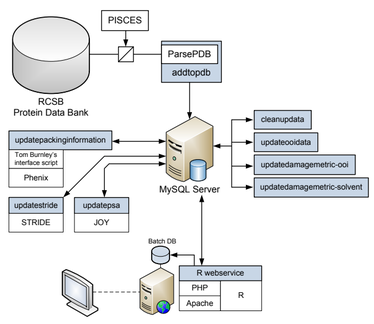
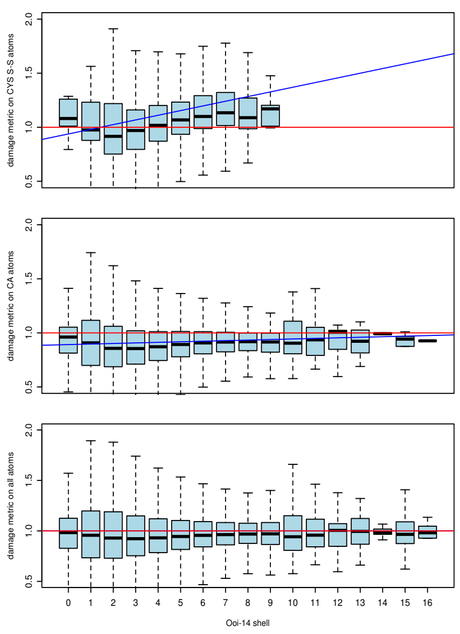
To investigate the effects of the atomic environment on specific damage we imported the atomic coordinates of a subset of the PDB into a local SQL database. Queries are executed against the database. Graphical output and reports for the investigator are generated using an R webservice.



Above: Overview of the used software. Blue boxes indicate software specifically developed for this work.

Results so far suggest that specific damage is distributed independently of secondary structure, solvent accessibility, protein residue count and disulphide bond configuration. Neighbouring amino acids and packing density of the residue show some correlation with damage distribution.



Left: Analysis on the relative uncertainty of atomic positions in proteins compared to all other atoms in equally dense packing environments. The Ooi-14 shell on the x-axis is an indicator of packing density.

Top: Densely packed disulphide sulphur atoms stand out against their environments.

Middle: Localization of Cα atoms is above average and independent of packing density.

Bottom: Reference graph of all atoms.

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With knowledge on the distribution of specific damage we can infer causes in the local environment. Given the three-dimensional structure of a protein we may then be able to predict sites that are particularly prone to specific radiation damage.

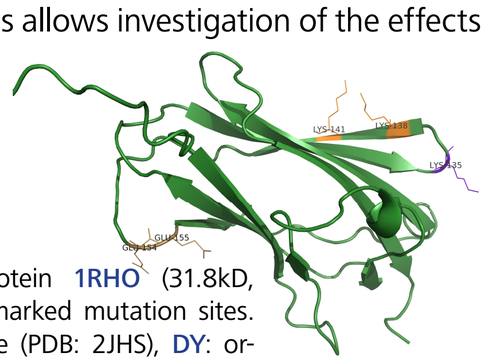
Hypotheses based on the statistical analysis of PDB subsets may be verified by appropriate experiments. Equally, experimental findings can be tested against the PDB.

improves understanding

has effects on

Experimental Investigation

Z.S. Derewenda (University of Virginia) kindly provided us with CH, EA and DY mutants of human signalling protein inhibitor (rhoGDI). These proteins have a high sequence identity but crystallize in different space groups. This allows investigation of the effects of a changed local environment on specific radiation damage patterns.



Original rhoGDI protein 1RHO (31.8kD, 139 residues) with marked mutation sites. CH: orange & purple (PDB: 2JHS), DY: orange (2JI0), EA: brown (2JHU)

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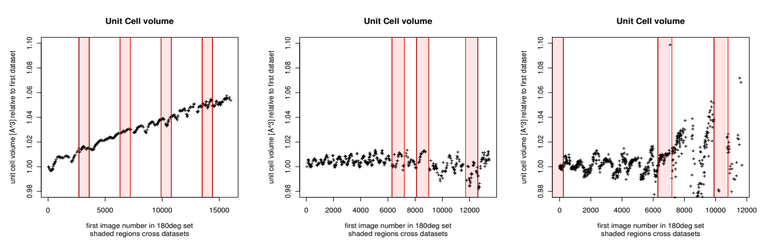
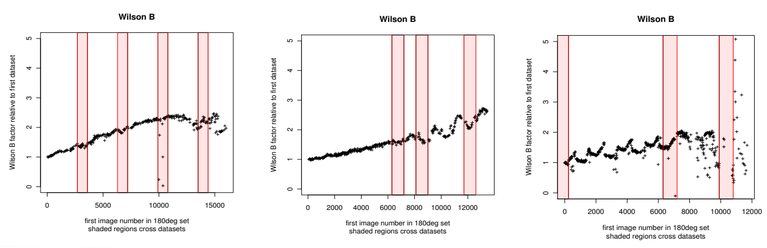
RhoGDI crystals were grown in multiple conditions, soaked in a cryoprotective solution and then cooled to cryotemperature. Experiments were conducted at Diamond Light Source, beamlines I03 and I04-1. Data were collected for full 360° wedges with no rotation axis offset and no translation at 100 K.

To track damage progression subwedges were randomly sampled and processed independently.



Above: Unit cell volume and Below: Wilson B factor for 180° datasets of three crystals relative to the first dataset.

Left: Unit cell grows over time, after linear growth Wilson B seems to peak. Middle: Unit cell volume does not increase, Wilson B oscillates in late datasets. Right: Increasing variance and inconclusive behaviour of unit cell size and Wilson B.



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These experiments may illustrate radiation damage progression by comparing the damage process in three similar proteins and between datasets. Dose-indicators could be established that have predictive value for the dose state of one protein crystal or all crystals of that particular protein.

validates

has effects on

It is conceivable to generalize these indicators into a general model for specific damage or dose-estimation. Insights could lead to improvements in phasing and provide a step towards refining structures to a zero-dose state.

Spatial Modelling

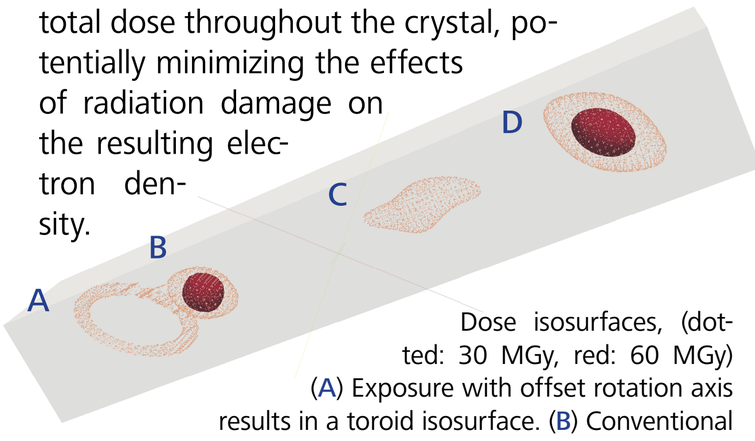
The parameter against which damage is quantified is dose (absorbed energy per mass, 1 Gy = 1 J kg^{-1}). The Henderson limit (20 MGy) predicts the upper limit for the global damage required to half the intensity of the diffraction pattern ($D_{1/2}$) for experiments at cryotemperatures. Biological fidelity of macromolecular structures is likely compromised at the Garman limit (30 MGy).

Keeping track of dose is an important consideration in crystallographic experiments. Yet dose can not be measured directly. The program RADDOSE can, given the experimental parameters, provide a dose estimate for experiments where the crystal is fully immersed in the beam.

▲ up to 2011 ▲ ▼ 2011 – 2013 ▼ ▲ up to 2011 ▲

With crystals larger than the beam and with inhomogeneous beam profiles, it becomes difficult to summarize the dose state of a crystal with just one number.

Instead of treating the crystal as an atomic entity RADDOSE-3D models the crystal as a voxel field. The accumulated dose is recorded for each voxel independently. When used for strategy optimization this allows a more even spread of the total dose throughout the crystal, potentially minimizing the effects of radiation damage on the resulting electron density.



Dose isosurfaces, (dotted: 30 MGy, red: 60 MGy) (A) Exposure with offset rotation axis results in a toroid isosurface. (B) Conventional data collection strategy with the same total dose results in a much higher maximum. (C) A helical scan provides a better dose distribution than (D) the conventional strategy.

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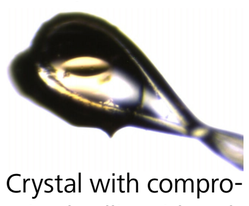
RADDOSE-3D was designed for extensibility. The next steps are to accommodate experimental beam profiles, experimental beam wavelength distributions, and the true 3D crystal shape.

When used during the experiment, RADDOSE-3D can become part of the strategy decision and assess strategies by their dose-distribution properties.



Crystal with compromised ellipsoid volume in the centre.

When a crystal is used in multiple exposures RADDOSE-3D can keep track of the dose levels of all crystal regions.

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