Quantitative Radiation Damage Studies in MX

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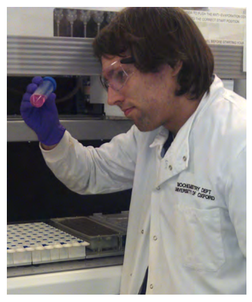
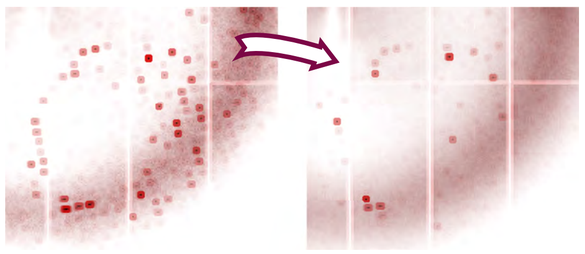


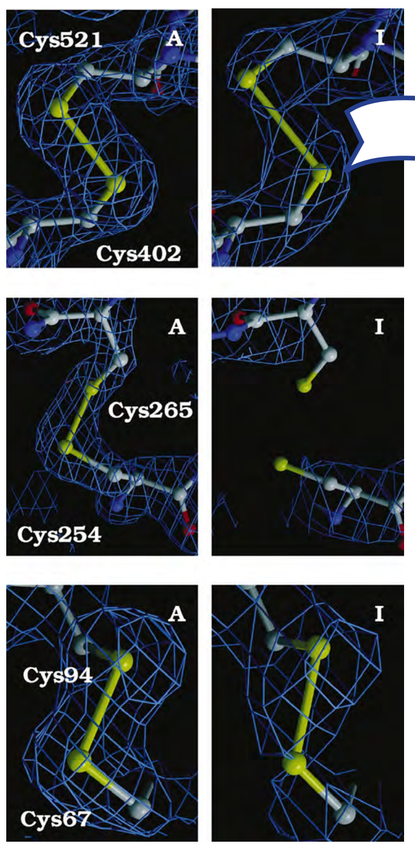
Specific Structural Damage

Radiation damage is a limiting factor in macromolecular X-ray crystallography diffraction experiments.



Global damage (**above**) can be observed during data collection even at 100 K as a decay of the diffraction pattern (highlighted in red) leading to a loss of high resolution information. The loss of long range crystal order leads to non-isomorphism.

Specific damage (**right**) causes detectable changes in the protein structure [1–3], such as the reduction of metallo-centres, breaking of highly electroaffinic disulphide bonds and decarboxylation of aspartate and glutamate residues. This leads to misleading biological conclusions on protein mechanism and function being drawn.

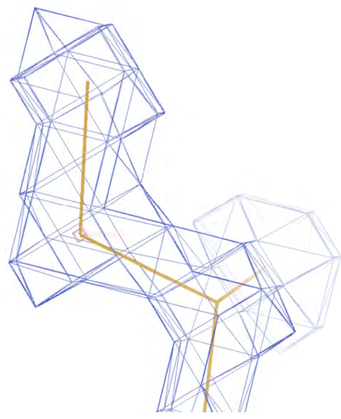


Disulphide bonds of *Tc*AChE breaking at different rates. First (**A**) and ninth (**I**) data set, 1.5σ, single crystal at 100 K, [3]

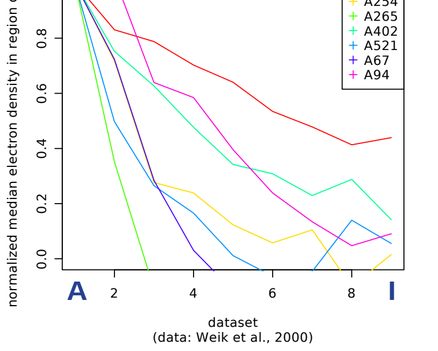
Real Space Electron Density Decay

Aim: Identify and quantify the susceptibility of protein residues to specific radiation damage.

Method: Obtain multiple data sets from one crystal (100 K). Using the structure determined with the first data set (**A**) as a reference, define the three dimensional space occupied by each residue (**right**). Calculate the relative median electron density in that volume for each subsequent data set. The electron density of susceptible residues will decay faster.

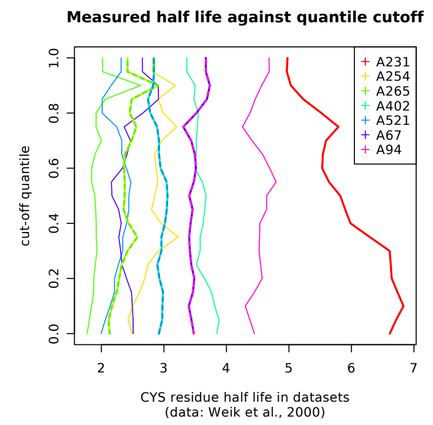


Three-dimensional volume of interest for one ILE residue of the reference structure.



Validation:
Using the *Tc*AChE data (**far left**) the order of the disulphides breaking can be reproduced by observing the electron density decay in their Cysteines (**left**)

Optimization:
The best separation (**right**) was obtained with a larger volume around each residue, constrained only by its neighbours and B factor.



'Residue half-life' determined by using smaller reference volumes. S–S bonds (defined as mean of CYS-medians) shown as two-coloured lines.

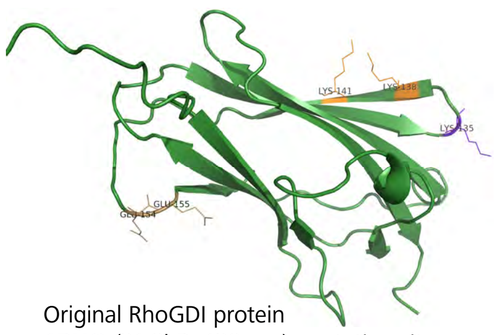
Quantifying Radiation Damage

We want to find a **causal connection** between physico-chemical parameters and preferential sensitivity. We present two different approaches for quantifying radiation damage: a **statistical analysis** of the Protein Data Bank [10] (**below**) and the tracking of electron density throughout a **crystallographic experiment** (**top right**).

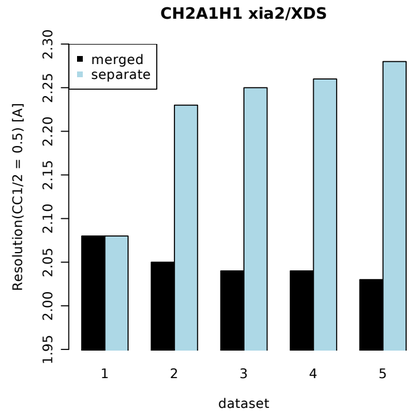
Experimental Investigation

We observe specific radiation damage to three RhoGDI protein mutants (**below left**) obtained by surface-entropy reduction [5–6]. These protein variants have a high sequence identity, but different crystal contact sites.

Experiments are ongoing. We aim to find differences in residue specific damage rates between these mutants.

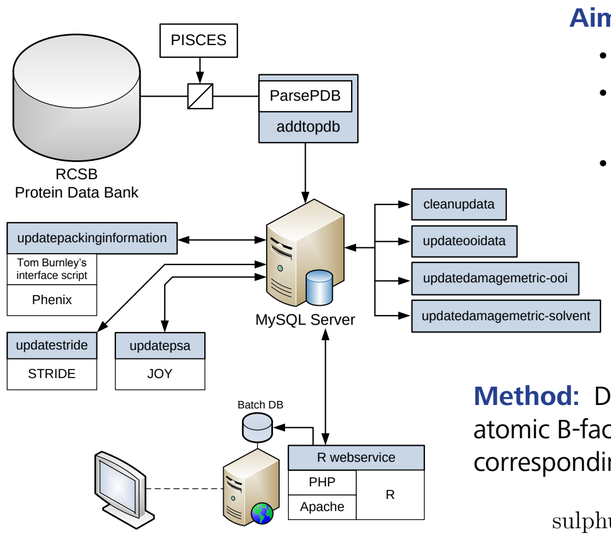


Original RhoGDI protein
1RHO (31.8kD, 139 res.); mutation sites:
CH: orange & purple (PDB: 2JHS),
DY: orange (2JI0), **EA**: brown (2JHU)



Left: CC½ = 0.5 resolution for five independent data sets of the same crystal (**light blue**) and merged data sets (**black**). Findings indicate CC½ is not a suitable metric to determine a cutoff point for compromised data, since merging compromised data sets will continuously improve the CC½ statistic.

Statistical Analysis of the PDB



Aim: Investigation of parameters such as

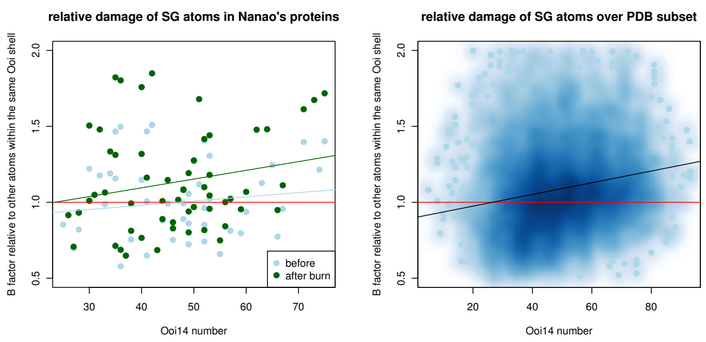
- solvent accessibility
- amino acid surroundings and packing density
- spatially and sequentially neighbouring residues

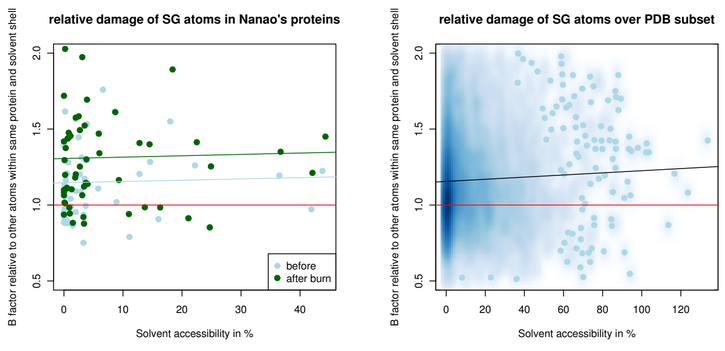
Method: Define a damage metric based on relative atomic B-factors stratified by the packing density of the corresponding residue in the protein environment, e.g:

$$\text{sulphur damage } (S_x) = \frac{B_{S_x}}{mean\left(B_{S(ooi=Ooi_x)}\right)} \quad [9]$$

Validation:
Using the metric with paired sets of good (**right**; **light blue**) and damaged (**green**) protein structures [4] results in appropriate and consistent behaviour.

Buried disulphides show higher relative damage than those on the protein surface (fitted line). A similar result can be observed in the PDB subset (**far right**).





Result:
While a correlation of sensitivity with packing density is observed (**above**), no correlation was observed with solvent accessibility (**left**).

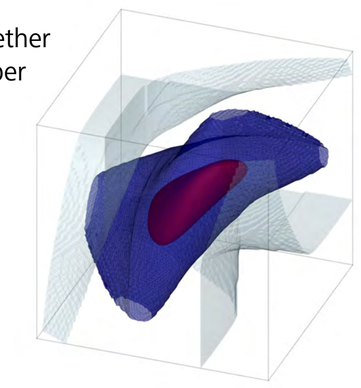
Limitations: Redundancy, annotation quality & consistency, optimised data [3], missing dose information, different experimental setups, data collection temperatures, …

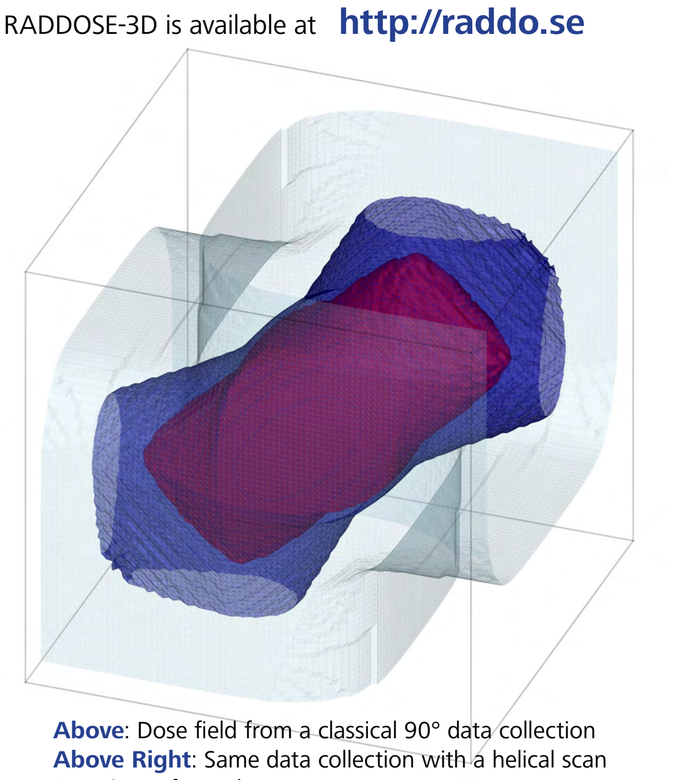
Results in Context: RADDOSE-3D

Pulling results, such as residue half-lifes, from different experiments together requires a common baseline. Describing a crystal state by a single number is nontrivial [8]. Any reliable metric has to be based on the **dose absorbed** by the crystal (Gy = J kg^{-1}), a quantity that cannot be measured.

RADDOSE-3D simulates diffraction experiments described by experimental parameters. It is the first software to provide dose estimates based on a 3D dose field, allowing for different dose regimes within the protein crystal [7].

RADDOSE-3D is available at **http://raddo.se**





Above: Dose field from a classical 90° data collection
Above Right: Same data collection with a helical scan
Dose isosurfaces shown at **0.1**/**20**/**30** MGy.

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