

# Understanding Specific Radiation Damage

Markus Gerstel<sup>1</sup>, Isaac Turner<sup>2</sup>, Charlotte M. Deane<sup>2</sup>, Elspeth F. Garman<sup>1</sup>

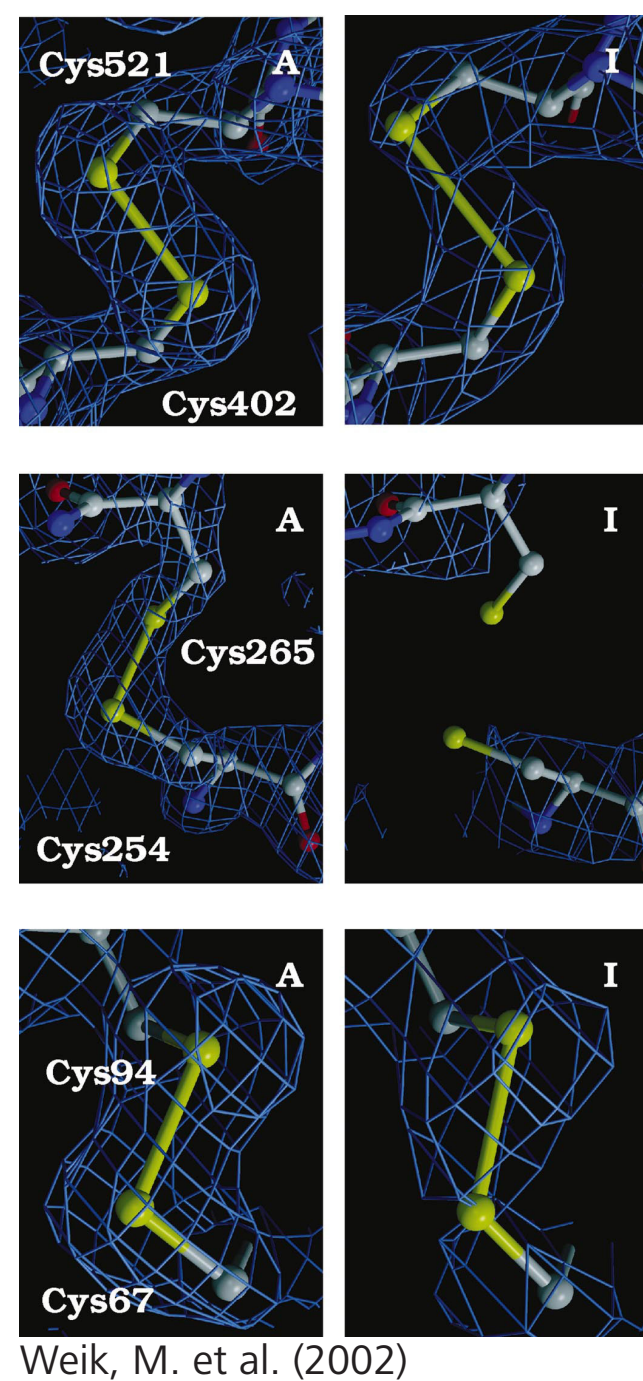
<sup>1</sup>Department of Biochemistry, <sup>2</sup>Department of Statistics, University of Oxford, South Parks Road, Oxford, OX1 3QU  
markus.gerstel@dtc.ox.ac.uk

## Specific Structural Damage

Disulphide bonds within a particular protein are especially sensitive to X-ray radiation. They are highly electro-affinic and act as a 'sink' for mobile electrons.

Weik et al., 2002 showed that the three disulphide bonds of *Torpedo californica* acetylcholinesterase do not break simultaneously, but in a defined order. Electron density comes from a series of data sets taken from a single crystal at 100K. The first (A) and the ninth data set (I) are shown to the right. In A all three disulphide bonds are intact. In the second data set (not shown) the Cys254–Cys265 disulphide bond is broken, in the fifth data set (not shown) Cys402–Cys521 is elongated by 30%, while Cys67–Cys94 still has its original length.

In the ninth data set (I) the Cys67–Cys94 bond length has still only increased by about 8%. The electron density of all three bonds has decreased. The contour is shown at 1.5 $\sigma$ .



Weik, M. et al. (2002)

## Data Mining of the PDB

Investigation of parameters such as

- amino acid surroundings and packing density (Ooi number, cx number)
- solvent accessibility (accessible surface area), disputed by Fioravanti et al. (2007), the only study on this subject so far
- local pKa
- spatially and sequentially neighbouring residues
- bond angles
- ...

## PDB Possibilities

Turner et al. (2010) proposed an estimate for specific radiation damage using atomic B factors:

$$\text{sulphur damage } (S_x) = \frac{B_{S_x}}{\text{mean}(B_{S(\text{ooi}=\text{Ooi}_x)})}$$

Sensitive substructures of the protein will be more disordered after X-ray exposure than more insensitive substructures. Here the substructure environment is taken into account by grouping residues with similar Ooi numbers. The Ooi number of a residue is defined as the number of C $\alpha$  atoms within a 14Å sphere around the residue's own C $\alpha$ . The Ooi shell is the Ooi number divided by 10, rounded down.

Thus by comparing the sulphur atomic B factor to B factors of all other protein atoms with similar packing density, it is possible to estimate radiation damage from a single PDB file.

The validity of using this metric as a measure of damage estimation was verified by Turner et al. (2010) against the Nanao et al. (2005) data set containing structures of 6 proteins, each before and after receiving a high dose X-ray burn.

## Relative damage progression

Our tests show (fig. A) that the relative atomic B factor of buried cysteine sulphur (higher numbered Ooi shells) is consistently higher than more exposed sulphur atoms. After the X-ray burn takes place a noticeable increase of relative atomic B factors can be observed in all Ooi shells.

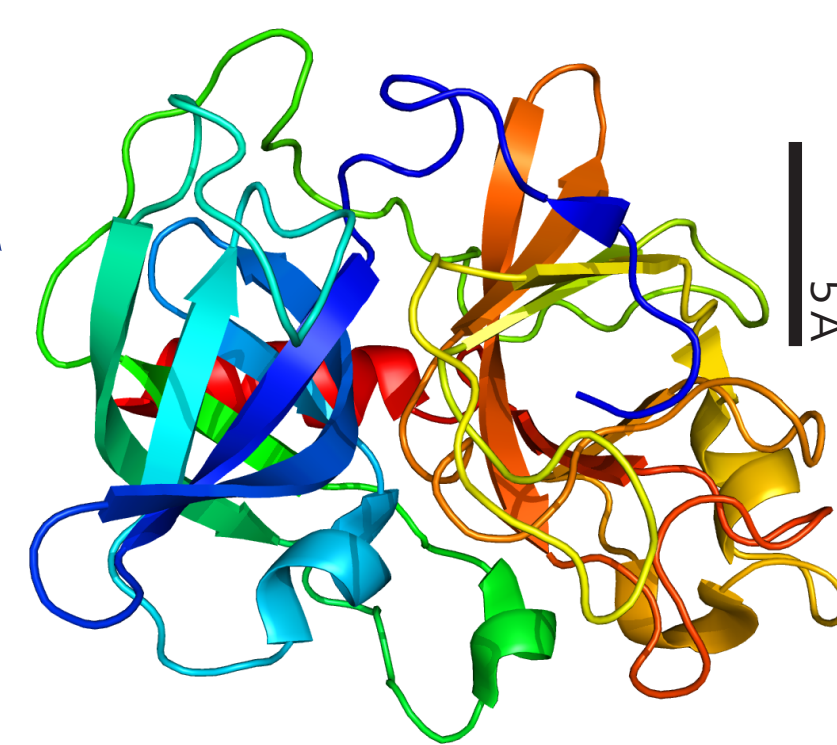
As a control we plotted the same graph for all C $\alpha$  atoms (fig. B), and observed that C $\alpha$  atoms in higher numbered Ooi shells have a relative B factor around 1. The X-ray burn has little effect on the C $\alpha$  population as a whole.

## Causes of Preferential Sensitivity?

We want to find a causal connection between physico-chemical parameters and preferential sensitivity of some disulphide bonds over others. Similarly, the radiation sensitivity of aspartate and glutamate residues is well known (Weik et al., 2000), and we want to explore preferential damage behaviour within groups of those residues.

We propose a two pronged approach: **Statistical analysis** of publicly available experimental data available through the PDB (Berman et al., 2000) and the Electron Density Server (Kleywegt et al., 2004) will be complemented by an **experimental approach** gathering radiation damage progression data.

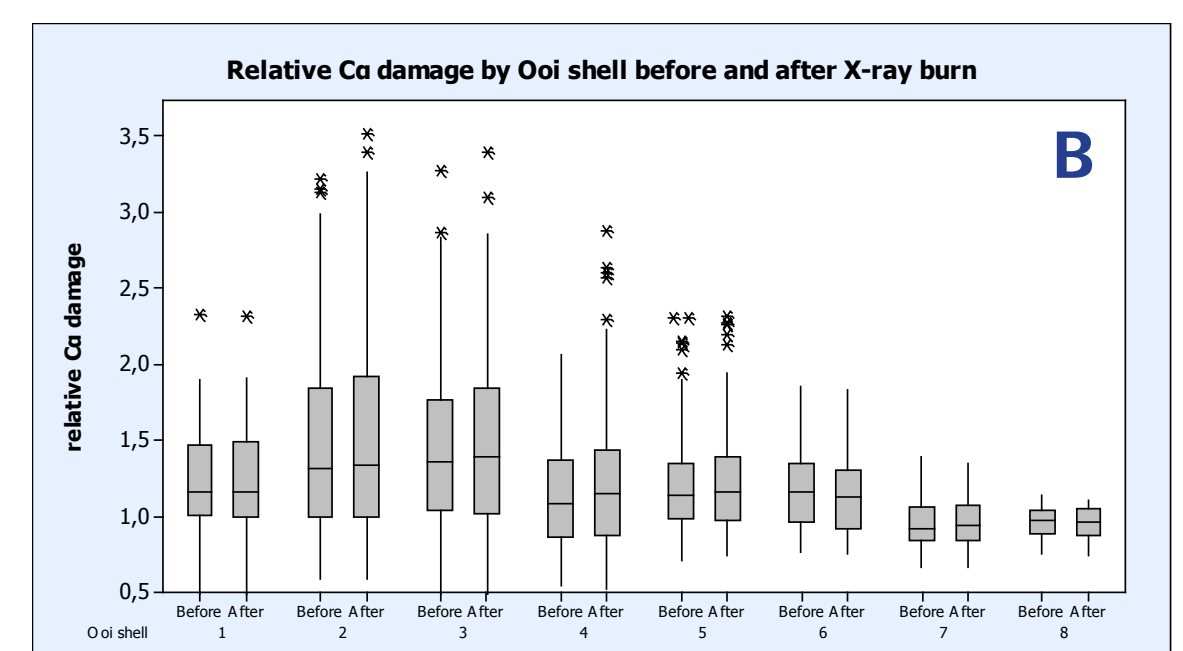
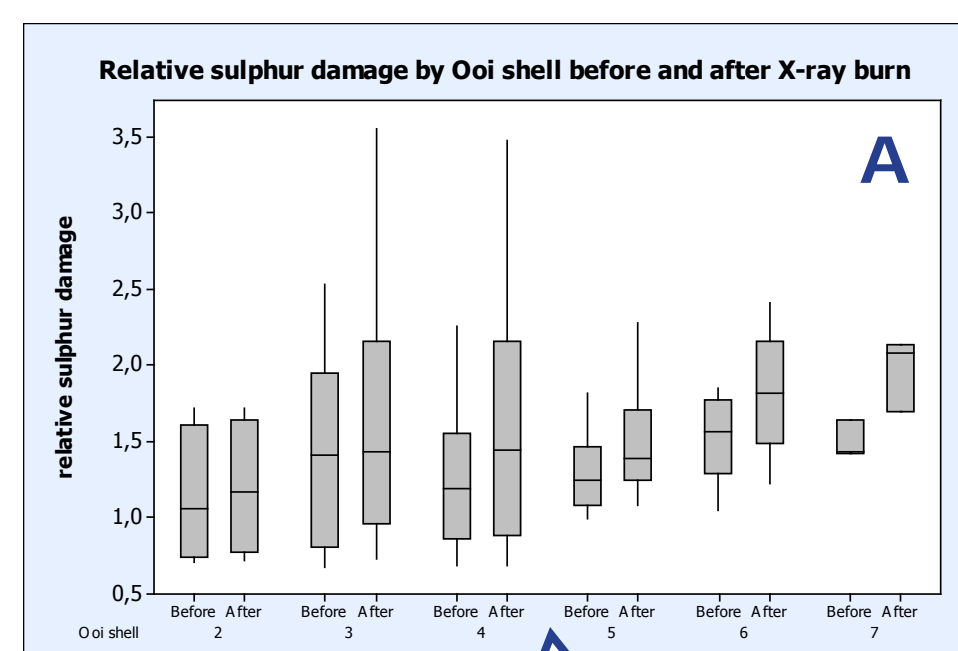
Statistical Analysis



2BLO: elastase before a high dose X-ray "burn"  
26.2kD, 240 residues,  
Nanao et al. (2005)

## PDB Pitfalls

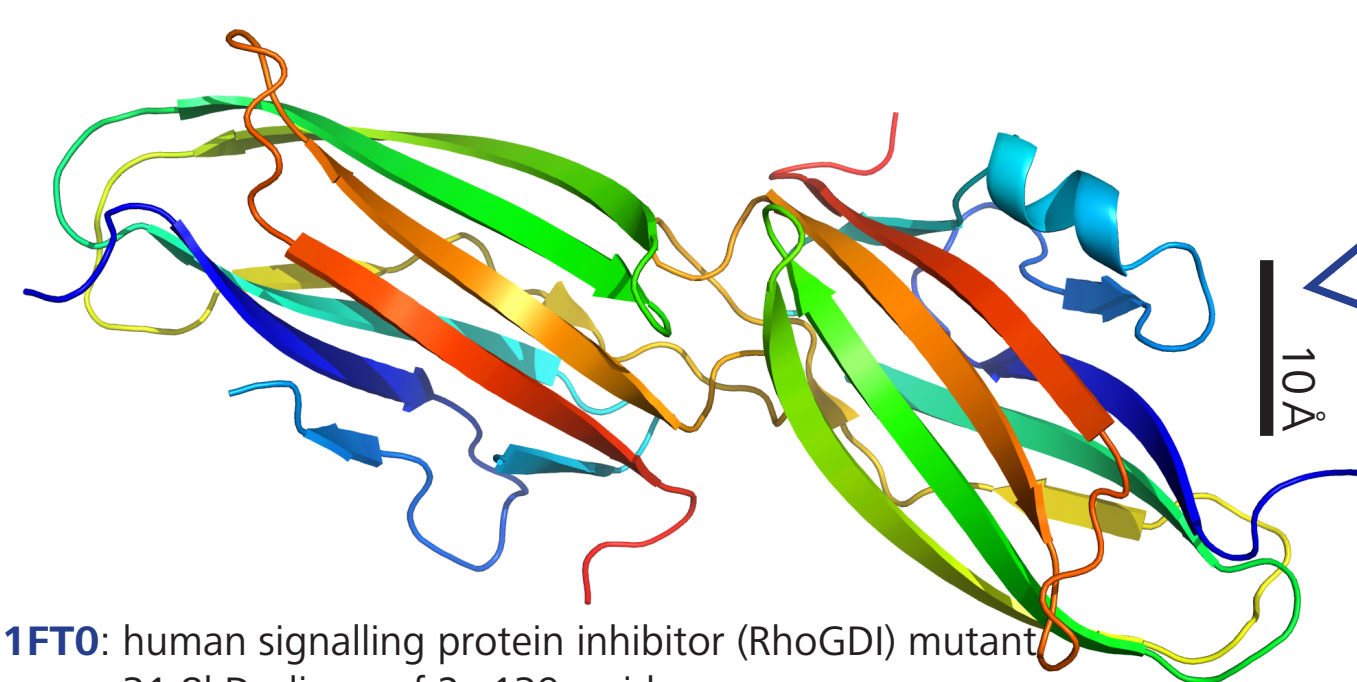
Redundancy, data annotation quality, inconsistent and missing annotation, working on optimised data, missing dose information, different experimental setups, undefined data collection temperature,...



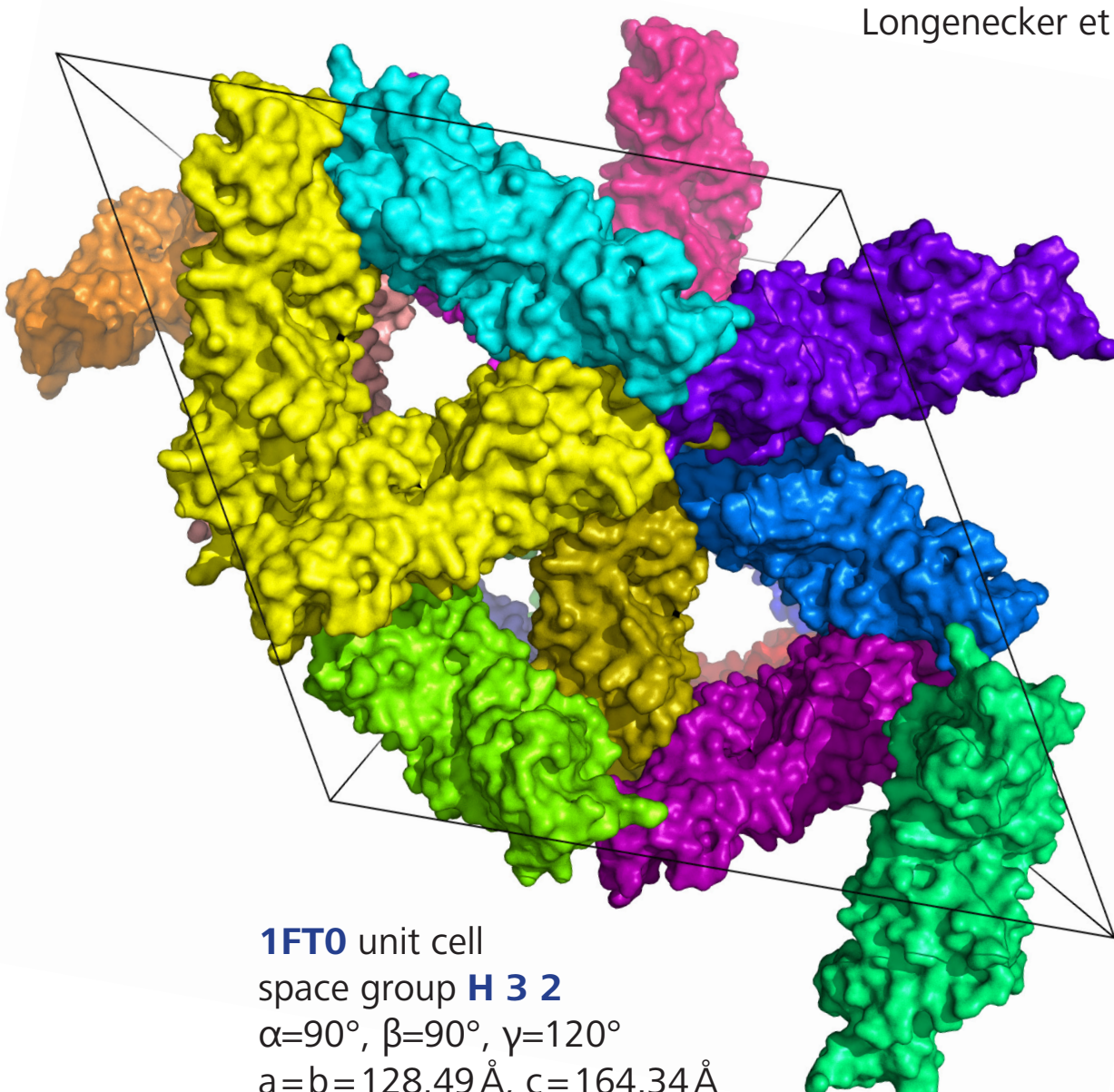
## Experimental Evaluation of Radiation Damage Progression

We will explore specific radiation damage on RhoGDI protein mutants, obtained by surface-entropy reduction (Derewenda, 2011). These protein variants have a high sequence identity, but different crystal contact sites.

Closely related mutants can crystallize in different space groups, allowing experiments over a wide range of solvent exposure scenarios.



1FT0: human signalling protein inhibitor (RhoGDI) mutant  
31.8kD, dimer of 2x 139 residues,  
Longenecker et al. (2001)



1FT0 unit cell  
space group H 3 2  
 $\alpha=90^\circ$ ,  $\beta=90^\circ$ ,  $\gamma=120^\circ$   
a=b=128.49Å, c=164.34Å

## Qualitative Analysis

What kinds of specific radiation damage progression can we observe? Are there other common, specific chemical degradation patterns?

## Quantitative Analysis

Can we quantify and model specific radiation damage given a better understanding of preferential specific damage?

## Comparative Analysis

What effect do small, local differences in the structure have on specific radiation damage? Can we predict highly sensitive substructures? Can we stabilise/destabilise substructures relative to the whole protein?

Synthesis

## Hypotheses

Predict order of specific radiation damage from structures or even sequences.

Experimental verification of hypotheses arising from PDB analysis. Statistical verification of hypotheses arising from experimental approach.

## Models

A predictive radiation damage progression model could be used to estimate the sequence and magnitude of specific structural changes. Ideally this model could be used to give an estimate for the dose to which a protein crystal has been subjected, aiding in one of the unsolved problems in macromolecular X-ray crystallography.

## Phasing, Refinement, Back-correction

An accurate radiation damage progression model could be used in Radiation-damage Induced Phasing (RIP) and refinement. It could be used to correct for specific radiation damage to some degree. Known structures could be examined for signs of specific radiation damage and – possibly – corrected to some extent.

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