Poster Presentations

[MS12-P03] Quantitative radiation damage studies in macromolecular X-ray crystallography Markus Gerstel[a], Natalya Olekhnovich[b], Jonathan Brooks-Bartlett[a], Zygmunt S. Derewenda[b], Charlotte M. Deane[c], Elspeth F. Garman[a]

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Radiation damage is a limiting factor in macromolecular X-ray crystallography diffraction experiments. Global damage leads to loss of long range crystal order, decay of the diffraction pattern and loss of high resolution information and non-isomorphism. Specific damage causes detectable changes in the protein structure [1-3], such as the reduction of metallo-centres, breaking of disulphide bonds, decarboxylation of aspartate and glutamate residues, and leads to misleading biological conclusions on protein mechanism and function being drawn. Two different approaches for quantifying radiation damage in MX are presented: a statistical analysis of the Protein Data Bank (PDB) and the tracking of electron density throughout a crystallographic experiment. The first approach applies a statistical model defining specific damage by changes in the distribution of relative atomic B-factors stratified by the packing density of the corresponding residue in the protein environment. This model was tested against a paired set of good/damaged protein structures [4]. A non-redundant subset of previously refined structures submitted to the PDB was then analyzed for indications of specific radiation damage. Results suggest that the distribution of specific damage is independent of secondary protein structure, solvent accessibility, protein residue count and isulphide bond configuration. There appears to be some correlation between the damage metric and the types of neighbouring amino acids. The power of an analysis of refined structures for signs of radiation damage is inherently limited: during refinement strong predictors of specific damage, such as the bond length of disulphides [3], will be optimized towards undamaged, theoretical values. The second approach to quantify radiation damage observes per-residue damage metrics based on the decay of real space electron density obtained from consecutive data sets compared to the first data set of the same protein crystal. The validity of the method is demonstrated by re-analyzing the specific damage data set of *Torpedo californica* acetylcholinesterase obtained by Weik *et al.* [1]. In our experiments we used human signaling protein inhibitor (rhoGDI) mutants [5] obtained by surface-entropy reduction [6]. These mutants have very high sequence identity, but, having different crystal contacts, readily crystallize in different space groups. Specific damage progression across different rhoGDI mutants can be compared by tracking the spatial dose distribution within each crystal and thus establishing the corresponding dose of the data sets using the newly available RADDOSE-3D [7].

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