# ESRP: INFLUENCE OF X-RAY ENERGIES ON RADIATION DAMAGE USING LYSOZYME AS A TOOL

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## *Introduction:*

Hen egg white lysozyme is one of the most studied proteins in biological crystallography. In this study, lysozyme is used as a model to investigate the effect of X-ray radiation damage on the structure of crystalline proteins. The advent of highly intense synchrotron beamlines has reintroduced the age-old problem of X-ray radiation damage in macromolecular crystallography, even for crystals held at cryogenic temperatures (100 K). Such damage to macromolecular crystalline samples during the experiment is an inherent problem when using ionizing radiation to obtain diffraction patterns and has presented a challenge to the crystallography field since the beginning.

One of the proposed analyses is to study the effect of radiation damage on disulfide bonds with respect to X-ray energies. Disulfide bonds contribute to stabilization of tertiary structure of lysozyme. Lysozyme has four disulfide bonds which consists of the two intra-α-domain disulfides, the intra-β-domain, and the inter-αβ-domains.

## *Experiment:*

The Hampton Research's Lysozyme Kit, containing a dozen 20 milligram vials of lysozyme and 12 milliliters of 0.02 M sodium acetate trihydrate pH 4.6 (solubilization buffer), was utilized to formulate the lysozyme crystals for the experiment. The buffer was pipetted into one lysozyme vial and was mixed gently to prevent denaturation. A final concentration of 20 mg/ml was formulated using 1,000 microliters of the buffer. Once the lysozyme crystals were obtained, they were frozen in liquid nitrogen using fomblin oil. All the samples were then loaded into the NE-CAT 24IDC beamline for data collection.

The data for tetragonal lysozyme was collected at 3 levels of energy: 7keV, 12keV, and 17keV. The NECAT 24IDC beamline is equipped with a Microdiffractometer-MD2 and Dectris Eiger2 X16M pixel array detector. The Dectris detector helps to collect relevant and accurate data in less time using the shutter-less data collection mode, thereby keeping crystal radiation damage to a minimum. Our analysis on radiation damage took this specific technological advance into account during the data collection. We utilized RAPD, HKL2000, XDS, and PHENIX programs for data processing, molecular replacement, refinement, and analysis. Additionally, we used COOT for model building, correcting the model, creating alternative conformations, and removing water molecules. We continued iterative refinement using PHENIX to bring down the R and Free-R parameters as low as possible.

**Figure 1:** Preparing lysozyme crystals.

## *Results/Discussion:*

| | 7keV | 12keV | 17keV |
|---|---|---|---|
| Space group | P 41 21 2 | P 41 21 2 | P 41 21 2 |
| a (Å) | 78.540 | 78.540 | 78.540 |
| b (Å) | 78.540 | 78.540 | 78.540 |
| c (Å) | 37.770 | 37.770 | 37.770 |
| α (°) | 90 | 90 | 90 |
| β (°) | 90 | 90 | 90 |
| γ (°) | 90 | 90 | 90 |
| Unit cell volume (Å$^3$) | 232985 | 232985 | 232985 |
| Data multiplicity (%) | 5.0 | 6.5 | 6.8 |
| Resolution limit (Å) | 50-2.1 | 50-1.25 | 50-1.1 |
| Bond lengths (Å) | 0.007 | 0.006 | 0.005 |
| Bond angles (°) | 0.777 | 0.782 | 0.774 |
| Number of protein residues | 129 | 129 | 129 |
| R-work (%) | 19.3 | 19.7 | 19.8 |
| R-free (%) | 25.3 | 25.8 | 20.3 |
| R-merge (%) | 11.4 | 10.6 | 4.4 |
| Completion (%) | 89.1 | 97.1 | 97.1 |
| CC 1/2 | 0.881 | 0.892 | 0.892 |

**Table 1: Crystal Data, Parameters, and Statistics**

Initially, we expected the 7keV to show more damage than the 17keV because the size of the 7keV wavelength corresponds closer to the disulfide bonds. However, according to our findings, all four disulfide bonds throughout the three levels of energy do not show any significant damage. The lysozyme's apparent resistance to radiation damage may be due to one of several factors.

The lack of measurable damage may be a result of using a well diffracting crystal, a low intensity beam, and short radiation exposure times. Current research suggests that bond geometry has an effect on bond damage when exposed to radiation. The disulfide bonds of lysozyme may have a geometry that allows them to be more resistant to radiation damage in comparison to the disulfide bonds of other molecules (Bhattacharyya et al.).

**Figure 2:** Group discussion of experimental protocols.

**Figure 3:** Pipetting lysozyme solution into wells to begin crystallization process.

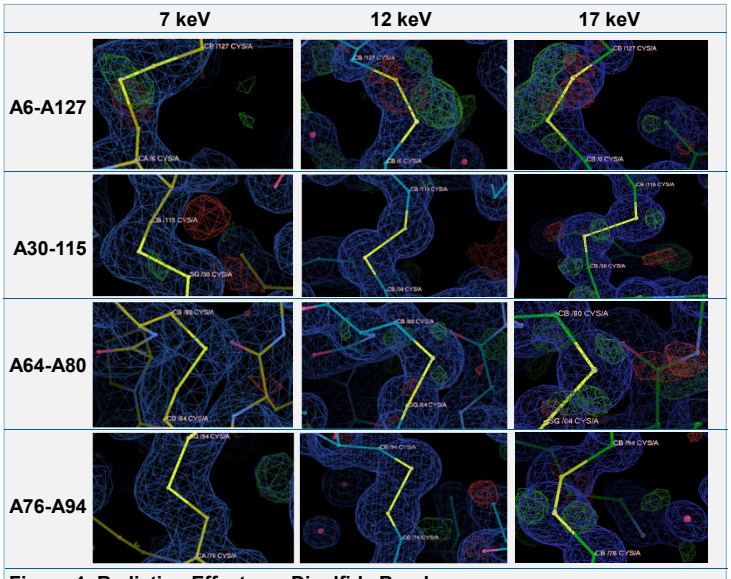


**Figure 4: Radiation Effects on Disulfide Bonds**

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