

# Analysis of Simultaneous Multiwavelength Anomalous Diffraction for Phasing Protein Crystals

Matthew A. Engel<sup>1,2</sup>, Marc Allaire<sup>2</sup>

<sup>1</sup>Department of Biomedical Engineering, State University of New York-Stony Brook, Stony Brook, NY 11794

<sup>2</sup>National Synchrotron Light Source, Brookhaven National Laboratory, Upton, NY 11973

**Abstract**— Macromolecular crystallography is a burgeoning field bridging the disciplines of biological chemistry, physics and material science. The diffraction of protein crystals is frequently required by pharmaceutical companies during the drug development process. Both intensity and phase of diffracted x-rays are required to solve a new crystal structure. Phases can be determined using the multiwavelength anomalous diffraction method (MAD). Simultaneous MAD (SMAD) has been proposed to significantly reduce the length of synchrotron data collection leading to faster structure determination. Analysis of MAD images collected at the peak and edge wavelengths reveal overlapping HKL reflections.

## I. INTRODUCTION

The reliance of cutting-edge biomedical research on synchrotron radiation is constantly expanding. The National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (BNL) maintains a source of hard x-rays for the user community by generating a stable flux of high energy radiation. Here, photons in the energy range of ~10keV may be used to observe the ultra-fine matter of biological and material samples. Suitable wavelengths for many biomedical experiments are near 1 Angstrom (Å), giving the observer access to data with subnanometer resolution.

The discovery of modern drugs often relies on information derived from x-ray crystallography and structural biology. These fields seek to determine the three-dimensional structure of proteins, nucleic acids and ligand-protein complexes at atomic resolution. A model produced by this method can reveal specific interactions at the ligand binding site, explain drug resistance, or lead to the development of rationally designed pharmaceuticals. Macromolecular crystallography has made a large imprint on biomedicine

and will continue to do so as high-throughput structural genomics expands. The current experiment will investigate the limitations of simultaneous multiwavelength data (MAD) data collection for protein crystal structure determination.

## II. MATERIALS AND METHODS

For this experiment, lysozyme (Sigma Co., US) crystals were used as a model system. Lysozyme crystals were grown by the hanging drop method using sodium chloride as a precipitant. Crystals were mounted on the goniometer head at the X6A beamline of the NSLS for data collection. One degree oscillation was employed throughout data acquisition. The images were recorded on an ADSC 210 detector. Data collection was performed at room temperature. Further experiments should be collected on cryogenically cooled specimens.

The wavelengths were selected from the Selenium (Se) Energy spectrum with a peak at 0.9792 Å, edge of 0.9795 Å and a remote of 0.9537 Å. A series of diffraction images were collected at each of these three wavelengths. Images were combined together using crystallographic software. Diffraction images at the first wavelength were retaken at the end of the experiment to assure that the reflections at that position were reproducible.

## III. RESULTS AND DISCUSSION

A protein's crystal structure cannot be solved without both a phase and intensity for each reflection in the diffraction pattern. Intensities are readily integrated over the number of photons which strike the CCD detector. However, regenerating the phase is not trivial. MAD allows one to reconstitute phase information, which cannot be directly measured during diffraction of native crystals. The scattering amplitude,  $f_j$ , for individual atom,  $j$  in a crystal can be represented as:

$$f_j = f^o + f' + if''$$

where  $f^{\circ}$  is the normal scattering and  $f'$  and  $f''$  are the anomalous scattering components. Resonant electronic transitions within the atomic orbitals cause x-ray perturbation. The principle of any MAD experiment is to take advantage of anomalous scattering of the heavy atoms by measuring signals at their peak, edge and remote wavelengths. After identifying the location of these heavy atoms in the macromolecule, phases can then be estimated.

In order to accelerate the data collection process, it has been suggested that simultaneous multiwavelength anomalous diffraction (SMAD) could be performed. The comparison of lysozyme crystal diffraction images obtained at the peak and edge (Fig. 1) wavelengths reveal identical diffraction. Diffraction images at the Se edge and remote wavelengths were found as a control. This suggests that the simultaneous collection of data from three wavelengths is not possible.

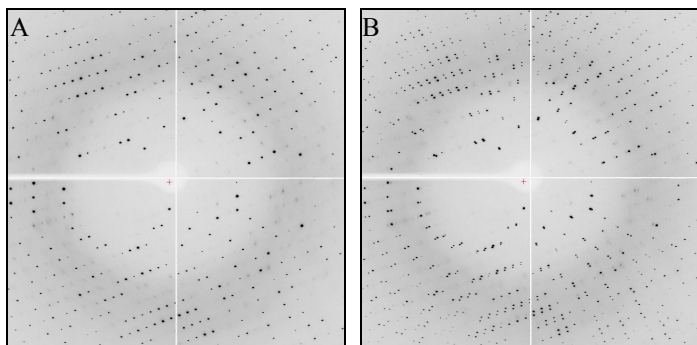


Fig 1. (A) Combined images of lysozyme crystals using MAD at the edge & peak creates overlapping diffraction patterns while (B) MAD of the edge & remote wavelengths are distinguishable.

Fig. 1 illustrates the overlapping diffraction patterns of lysozyme crystals collected from two different wavelengths. (A) Overlapping images of the peak and edge, separated by  $0.3 \times 10^{-3}$  Å look identical. Only MAD at the (B) remote and edge wavelengths were distinguishable. The lack of individual reflections from the peak and edge energies creates identical diffraction patterns. In comparison, data from the edge and remote wavelengths are resolved

#### IV. CONCLUSIONS

The crystallization of biological macromolecules is an integral technique in the rational design of pharmaceuticals. A rapid increase in the amount of proteins available for x-ray diffraction should

be accompanied by further improvements in synchrotron data analysis to support three-dimensional model building. MAD has become an established technique used to recover the phase information, easing this process. However, simultaneous MAD data collection has not been proven feasible.

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