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Strategies for native-SAD experiment at low X-ray energy: Users' Reports

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1 Introduction

Native-SAD (Single-wavelength Anomalous Diffraction) phasing is becoming the dominant method towards *de-novo* X-ray structure determination of biomolecules. This method relies on weak anomalous intensities from the light anomalous scatterers (*e.g.* S or P atoms or ions natively present in biomolecules). These light elements have X-ray absorption edges in the region of 2-3 keV, which cannot be reached at most macromolecular crystallography beamlines. However, BL1A beamline at Photon Factory has a unique setup, where low X-ray energy can be achieved down to 3.7 keV. One of the major difficulties of native-SAD experiments at low X-ray energy is the high absorption. Here, long absorption X-ray path length is detrimental to the anomalous signal from S atoms. Presence of solvent in the loop, cryo-stream of liquid N_2 and air around the crystal can further outweigh the gain in anomalous signals. However, the diffraction efficiency of crystals is supposed to increase in the order of square of λ. It has been noticed that for crystal sizes smaller than 100-µm, use of low X-ray energy can increase the anomalous signal from S atoms. Fig. 1a shows the calculated anomalous signals for different crystal size and X-ray wavelengths, in absence of any absorption effect.

In this work, we performed native-SAD measurement on crystals of various thicknesses with aim at improving data quality by minimizing the X-ray absorption effect at low X-ray energy.

2 Experimental setup and Data collection

We devised an experiment, where we tried to achieve the ideal sample preparation for experiment at low energy. We used lysozyme crystals of various sizes mounted on Micro-acti-loop from MiTeGen with reduced amount of solvent. The crystals were mounted on a goniometer, cryo-cooled and collected at 4.59 and 3.75 keV X-ray energies.

For native-SAD measurement, we first normalized the transmissions of different X-ray wavelengths (2.7 and 3.3 Å) using a long 50 µm shaped lysozyme crystal. A V-shaped detector configuration with two EIGER X 4M detectors was used with a tilt angle of 25° (See Fig. 1b) in order to catch high-angle reflections at 3.3 Å (3.75 keV). We used 50x50 µm beam. BL-1A is equipped with a He-chamber to reduce air absorption. We used 3.04% and 2.27% transmissions at 2.7 and 3.3 Å X-ray wavelengths respectively. Datasets were collected with oscillation wedge of 0.2° and exposure time of 0.1 sec.

3 Results and Discussion

Successful native-SAD measurement implies high data quality and high anomalous signal while keeping high-angle reflections. The V-shape detector configuration provided highest resolution of 2.4 Å compared to the flat configuration which could provide resolution only up to 3.4 Å at 3.75 keV. This improvement in resolution was important for successful substructure determination at 3.3 Å X-ray wavelength (3.75 keV energy). BL1A experimental setup allows the reduction of absorption effects at low X-ray energy. So, comparison of anomalous signals (Fig. 1c) attained at 2.7 Å (4.59 keV) and 3.3 Å (3.75 keV) X-ray wavelengths shows that longer wavelength native-SAD could indeed produce higher anomalous signals. As a result, gain in anomalous signals could be utilized for successful phasing of lysozyme and structure could be determined at 3.75 keV (Fig. 1d). This study helped us to understand optimum crystal size for long-wavelength data collection.

After successfully establishing the strategy with lysozyme crystals, we want to apply it to real-life challenging proteins crystals.

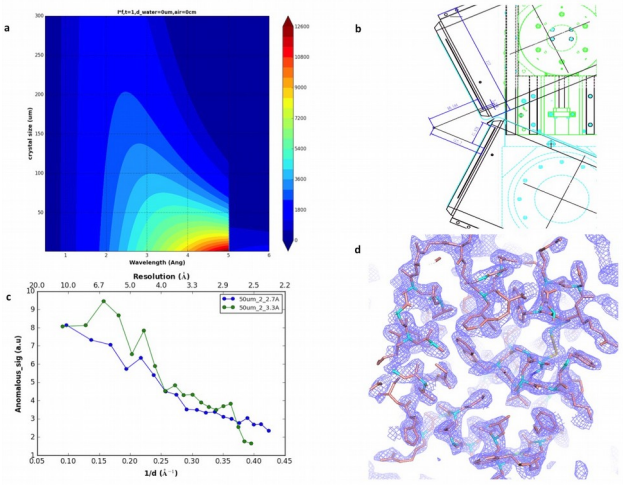

Fig 1. Ideal native-SAD measurement, experimental setup, and electron density at 3.3 Å (or, 3.75 keV) X-ray wavelength. 1a. It shows a 2-D contour plot for optimal anomalous intensity for a given crystal size and X-ray wavelength in ideal condition (i.e., no absorption effect) based on a simulation. **1b.** It represents the V-detector configuration at BL-1A beamline and

experimental setup. **1c.** It compares the anomalous signals between two X-ray wavelengths – 2.7 and 3.3 Å based on data collected on the same 50 μm shaped crystal. **1d.** The electron density of lysozyme crystal contoured at 1.5 σ, proving successful native-SAD structure determination at 3.3 Å X-ray wavelength.

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References

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