Influence of Crystal Size and Wavelength on Native SAD Phasing

The native SAD method, an emerging phasing method for protein crystallography, typically utilizes low energy X-rays to enhance anomalous signals from sulfur atoms in the protein. It has been hypothesized that shorter wavelengths should be used for large crystals and longer wavelengths for small crystals to minimize the absorption of the low energy X-rays. However, no experimental analyses have been performed to investigate this hypothesis. Our systematic experiments suggested that large sample sizes do not have detrimental effects on native SAD data and longer wavelengths are more advantageous than shorter wavelengths for use with small samples. The individual sample quality and crystal properties seemed to have much more influence than the wavelengths.

Native SAD is an emerging phasing technique using anomalous signals from native proteins. Typically sulfur atoms in the proteins have been utilized as anomalous centers. Since the native SAD method does not require a procedure to introduce heavy atoms in the protein crystal, it is expected to be one of the best methods for the crystal structure determination of proteins. However, the native SAD method requires high quality diffraction data due to the weak anomalous signals of sulfur atoms. Long-wavelength X-rays are typically utilized to enhance the anomalous signals in native SAD experiments, but this solution results in more background noise and sample absorbance than usual, leading to a degradation of data quality [1, 2]. This trade-off between the requirement of high quality diffraction data and the final data quality has discouraged use of the native SAD method for routine crystal structure analysis. To overcome these difficulties, we have constructed a beamline for the native SAD method, BL-1A. In BL-1A, the goniometer and X-ray detector are placed in a helium chamber to reduce background and absorption in data collection. Moreover, users can utilize a small beam (13 μm x 13 μm) with a pixel array-type detector for low-noise data collection. While these experimental setups are suitable for the native SAD method, the crystal itself would still hamper high quality data collection due to its absorbance of the long wavelength X-ray. It has been hypothesized that large crystals should be probed with shorter wavelengths to decrease absorption, while small crystals, for which absorption is not very significant, should be analyzed with longer wavelengths to benefit from the increased anomalous signals [3]. While such an approach is theoretically reasonable, there is currently no experimental evidence to support it. In this study, therefore, we attempted to establish a general protocol for the native SAD method by analyzing the relationship among wavelength, crystal size, and data quality using BL-1A and BL-17A. BL-17A is another beamline frequently utilized for data collection with relatively long wavelength [4].

Experiments were performed under helium environments with lysozyme and ferredoxin reductase (BphA4) crystals of dimensions 50 – 300 μm. Initially, we examined native SAD phasing at wavelengths of 1.9 and 2.7 Å. Where possible, each crystal was probed with two wavelengths to minimize the influence of a particular sample on the quality of the diffraction data (**Fig. 1**). For medium and small BphA4 crystals, the data sets collected at 2.7 Å wavelength clearly provided better phasing results than those collected at 1.9 Å wavelength. For lysozyme, the differences between wavelengths were subtle. The substructure search was marginally more effective at 1.9 Å for large crystals, whereas it was similarly effective at both wavelengths for medium crystals. For small crystals, data collection at 2.7 Å clearly provided the best results for the substructure search. Finally, for all crystal sizes, phasing and density modification yielded marginally better phases at 2.7 Å.

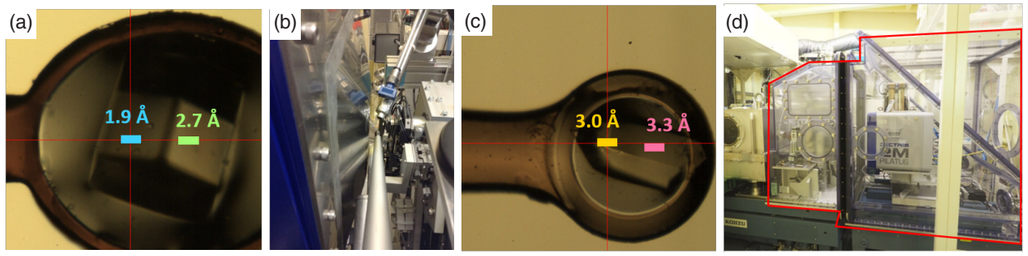

Figure 1: (a) A lysozyme crystal used in an experiment with X-rays of 1.9 and 2.7 Å wavelengths. The beam size used in the experiments was 2.5 x 10μm^2, as indicated by rectangles in the figure. **(b)** A helium-cone at BL-1A. **(c)** A BphA4 crystal used in an experiment with X-rays of 3.0 and 3.3 Å wavelengths. The beam size used in the experiments was 2.5 x 10μm^2, as indicated by rectangles in the figure. **(d)** A helium-chamber at BL-1A.

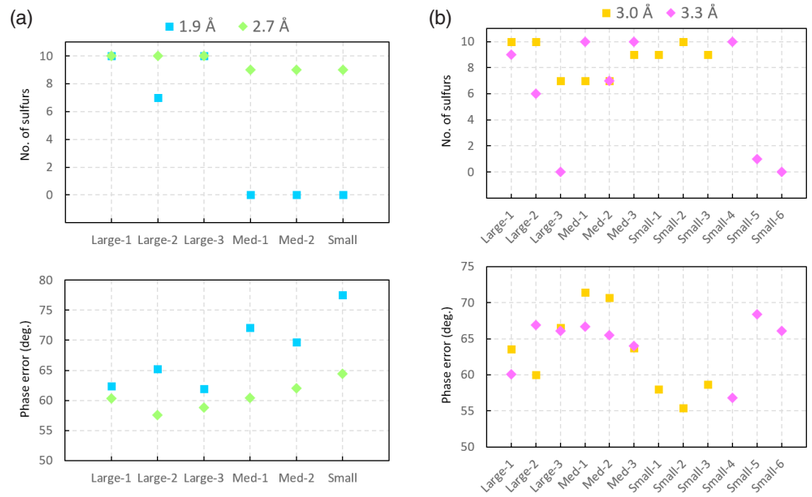

Figure 2: Effects of data-collection wavelengths and crystal size on native SAD phasing. **(a)** Data for large, medium and small BphA4 crystals collected at 1.9 Å (blue) and 2.7 Å (green). **(b)** Data for large, medium and small BphA4 crystals collected at 3.0 Å (yellow) and 3.3 Å (magenta). The upper charts for both **(a)** and **(b)** show the number of sulphur atoms found by SHELXD at the four data-collection wavelengths, and the lower charts show their corresponding average phase errors after density modification.

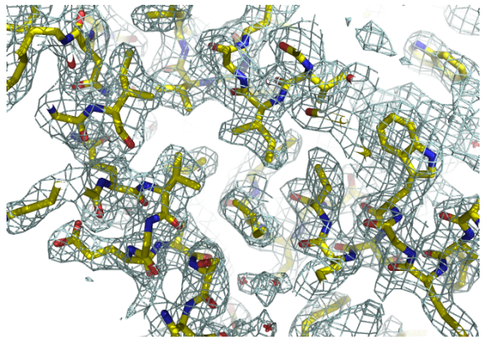

Figure 3: An electron density map at 2.5 Å resolution obtained by native SAD method.

Next, we compared the results of native SAD phasing with X-ray wavelengths at 3.0 and 3.3 Å, and found that phasing worked equally well at both wavelengths for the lysozyme and BphA4. Compared to the 1.9/2.7 Å wavelengths, the 3.0/3.3 Å wavelengths were more advantageous for medium and small BphA4 crystals (**Fig. 2**). Even in the case of large crystals, however, which were expected to suffer from an absorption problem, it was possible to determine the substructure and obtain high quality phases comparable to those from small crystals. Thus, the individual sample quality and crystal properties seemed to have much more influence than the wavelengths.

Based on the results of this systematic analysis, the Structural Biology Research Center in IMSS initiated to support users for native SAD experiments. In conjunction with this initiative, several users have already succeeded in the crystal structure determination of proteins using the native SAD method [5-8]. Moreover, a combination of the molecular replacement and native SAD methods, the MR-Native SAD method, is becoming a powerful tool for fast protein-structure determinations. The high quality experimental phases obtained from the MR-native SAD method (**Fig. 3**) are expected to accelerate the speed of crystal structure determinations.

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BEAMLINES

BL-1A and BL-17A

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