

STUDIES ON THE MAD METHOD AT THE RIKEN BEAMLINE I BL45XU

The introduction of synchrotron radiation has accelerated the accumulation of three-dimensional structures, and with the tunability over a wide energy range of the synchrotron radiation, the multi-wavelength anomalous diffraction (MAD) method, which supplies phases from a single anomalous scatterer [1], has been developed [2]. By utilizing the third generation SR with the maximized advantage of the MAD method, **RIKEN beamline I (BL45XU)** is designed to contribute to research on structural biology.

One of the critical problems in macromolecular crystallography is the phase problem. The multiple isomorphous replacement (MIR) method has been the most useful phasing method in macromolecular crystallography. However, the MIR method requires at least two different heavy atom derivatives, of which crystals have to be in a high degree of isomorphism with the native crystals. On the other hand, employing the MAD method, all the data can be collected from the same crystal, if it contains anomalous scatterers. The MAD method has the advantage in terms of both accuracy and convenience in phase evaluation.

However, the development of the MAD method as a routine method in macromolecular crystallography is not straightforward because the contribution by the anomalous scatterer is minimal. To ensure accuracy in MAD experiments, it is essential to minimize systematic errors, such as absorption, detector characteristics and radiation damage, so that actual signals in Bijvoet and dispersive differences are measured as precisely as possible. In MAD experiments, at least three sets of diffraction data have to be collected with different wavelengths. In addition, the wavelengths have to be tuned as quickly as possible with a good reproducibility.

To achieve such an experimental environment for the MAD method, the "trichromatic concept" was introduced by the development of high-quality diamond crystals [3]. The trichromatic concept maintains that three kinds of data sets at three wavelengths are taken quasi-simultaneously for the same protein crystal without changing the setting by

the "trichromator". The trichromator consists of three pairs of transparent diamond double-crystal monochromators with a fixed exit; it collinearly introduces X-rays with three monochromatized wavelengths in an identical beam direction (Figure 1).

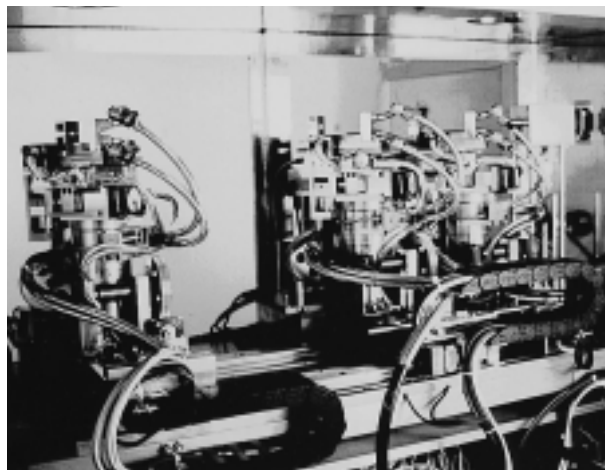


Fig.1: A sideview photograph of the trichromator. The trichromator contains 3-pairs of diamond double-crystal monochromators. Courtesy of IUCr [4].

Dichromatic synchrotron radiation is emitted from tandem vertical undulators. Monochromatized radiations of three wavelengths are cut out from the dichromatic synchrotron radiation with the trichromator, and the three are sequentially supplied through beam choppers. The data collection is sequentially performed at each wavelength to minimize systematic errors as well as background interference produced by other wavelengths (Figure 2).

The construction of the **RIKEN beamline I (BL45XU)** was started in August 1996, and progressed until June 1997. The commissioning of the beamline with synchrotron radiation began in July 1997. In the initial test, the trichromator successfully monochromatized three wavelengths at the same time, and three undulator beams were observed [4]. In the experimental station, a four-circle diffractometer and two different types of two-dimensional detectors were arranged. As a fast read-out two-dimensional detector, a multiple CCD X-ray detector (MCCDX) is being developed in order to record the diffraction patterns of protein crystals [5]. The MCCDX detector will have a 4 x 4 matrix

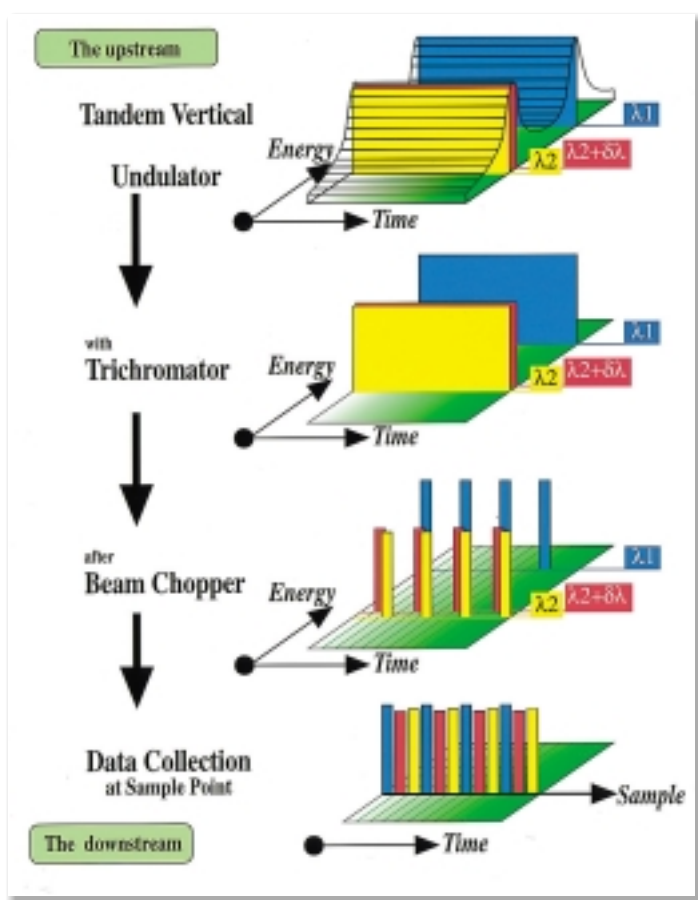


Fig. 2: Outline of the “trichromatic concept”. Courtesy of IUCr [4].

array of CCD X-ray detector modules with an active area size of 200 x 200 mm². In the initial stage, an imaging plate detector (RIGAKU R-AXIS IV) was installed and used for MAD data collection.

For MAD data collection, the first pair of the trichromator is set at a remote point of the absorption edge to obtain an anomalous scattering-free data set. As a result of XANES profile measurements, the other two wavelengths are supplied at the peak and edge energy of the anomalous scatterer, to maximize anomalous scattering contributions. The beam chopper sequentially supplies one wavelength out of the three to the experimental station.

The crystal of blasticidin S deaminase (BSD) was analyzed as the first sample. BSD is an enzyme which includes one Zn atom per one molecule (*Mr* ~ 13,000), and a homo-tetramer of BSD is included in an asymmetric unit. Figure 3 shows the

XANES spectrum of BSD. The remote, peak and edge wavelengths of the Zn atom were selected at 1.0 Å, 1.2818 Å and 1.2822 Å, respectively. Three wavelength data sets of diffraction images were collected at 2.2 Å resolution. Four Zn positions of the asymmetric unit were clearly shown in anomalous and dispersive difference Patterson maps, and the initial electron density map was easily calculated. Figure 4 shows the MAD phased initial electron density map obtained, which clearly shows the secondary structure and side chains. The model building and the refinement of BSD progressed satisfactorily [6].

On the other hand, a dichromatic diffraction experiment using two wavelengths simultaneously was planned as a more efficient use of the trichromator. Two different wavelength diffractions at the remote and the edge of an anomalous scatterer were recorded on one imaging plate, and the diffraction at the third wavelength was recorded on another

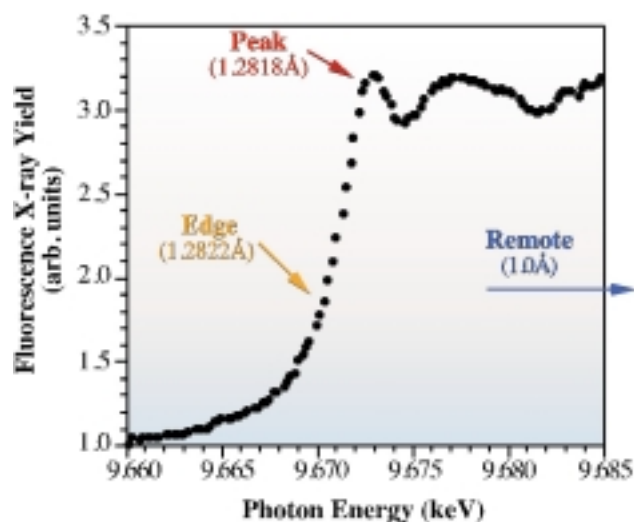


Fig. 3: The XANES spectrum of a BSD crystal, which includes a Zn atom. The MAD data collection was carried out at the wavelengths of 1.0 Å, 1.2818 Å and 1.2822 Å.

imaging plate. Figure 5 shows dichromatic diffraction images of another Zn protein. The dichromatic diffraction images were processed by using ordinary indexing software, the anomalous and dispersive difference Patterson maps clearly showed the Zn positions. The structural analysis progressed sufficiently. To date, we have already determined six structures, including one more Zn protein crystal, two Hg derivative crystals, and one Se-Met crystal.

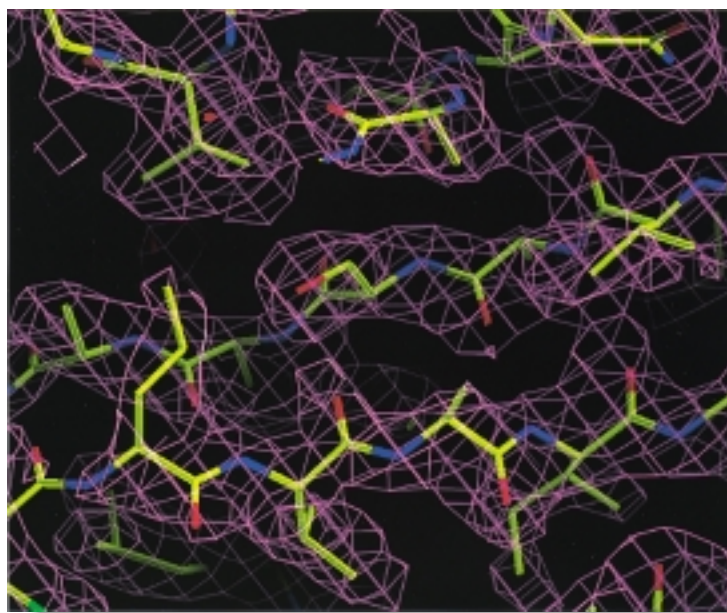


Fig. 4: MAD phased initial electron density map of BSD at 2.3 Å resolution.

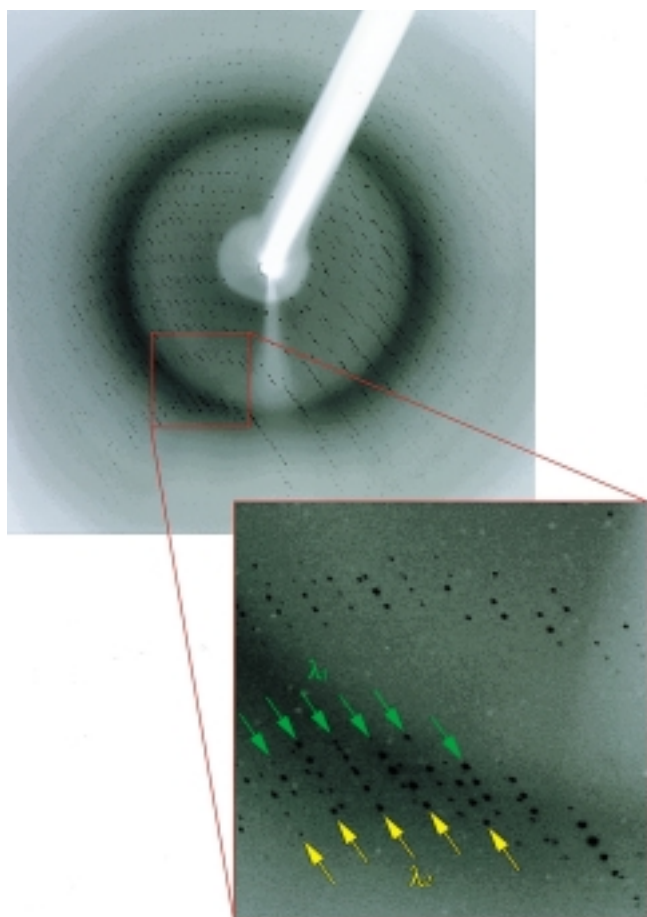


Fig. 5: Dichromatic diffraction images of a Zn protein. Two different diffractions at the remote (λ_1) and the edge (λ_2) wavelengths were exposed on the same imaging plate.

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