Acta Crystallographica Section A
Foundations of Crystallography
ISSN 0108-7673

Received 27 September 1999
Accepted 6 January 2000

Enantiomorph determination using inverse reference-beam diffraction images

Qun Shen,[a,b]* Stefan Kycia[a] and Ivan Dobrianov[c]

[a]Cornell High Energy Synchrotron Source (CHESS), Cornell University, Ithaca, NY 14853, USA, [b]Department of Materials Sciences and Engineering, Cornell University, Ithaca, NY 14853, USA, and [c]Department of Physics, Laboratory of Atomic and Solid State Physics, Cornell University, Ithaca, NY 14853, USA. Correspondence e-mail: qs11@cornell.edu

It is shown that enantiomorph structures of a noncentrosymmetric crystal can be determined, in the absence of anomalous diffraction signals, by measuring two series of reference-beam oscillation diffraction patterns related by an inverse-beam geometry. The corresponding intensities of the Friedel pairs recorded on the two sets of images exhibit the characteristic three-beam interference effects that provide the unambiguous phase information. The experimental arrangement and the data-analysis procedure are demonstrated through an experimental example on tetragonal lysozyme.

1. Introduction

It has been demonstrated recently (Shen, 1998, 1999*a*; Shen *et al.*, 1999) that a large number of three-beam interference profiles and the corresponding invariant triplet phases can be measured experimentally using a standard oscillation camera set-up in a Bragg-inclined reference-beam diffraction geometry (Fig. 1). This reference-beam diffraction (RBD) technique provides a convenient and practical way of collecting direct crystallographic phase information in a time frame similar to that of a multiple-wavelength anomalous diffraction (MAD) experiment (Hendrickson, 1991) but without the necessity to absorb heavy atoms in the structure. An important step in MAD data collection is to measure the intensities of the Freidel pairs, **H** and $-\mathbf{H}$, in the oscillation diffraction patterns, which allows for the determination of enantiomorphs in noncentrosymmetric crystals. In this article, we show that the same principle can be equally applied in reference-beam diffraction, where two sets of inversion-symmetry-related reference-beam diffraction patterns are measured to determine the signs of the triplet phases of a large number of Bragg reflections. We demonstrate this point with a new series of data collected in the inverse-beam geometries using a charge-coupled-device (CCD) X-ray detector on a tetragonal lysozyme crystal.

2. Theory and experiment

In a typical RBD experiment (Fig. 1), a strong reference Bragg reflection **G** from the crystal is excited and aligned along the crystal-oscillation axis. The integrated intensities of many other reflections, **H**, are collected on an area detector by oscillating the crystal through the same rotation range $\Delta\varphi$. The data collection is repeated multiple times, each time at a slightly different rocking angle θ around the Bragg angle $\theta_{\mathbf{G}}$ (Shen, 1998, 1999*a*). The intensity profile, composed of these multiple exposures as a function of θ, $I_{\mathbf{H}}(\theta)$, contains the information on the triplet phases of the involved structure factors owing to the principle of the three-beam interference effect (Hart & Lang, 1961; Colella, 1974; Post, 1977; Chapman *et al.*, 1981; Chang, 1982; Juretschke, 1982; Tischler & Batterman, 1986; Shen, 1986; Shen & Colella, 1987; Shen & Finkelstein, 1990; Chang *et al.*, 1991, 1999; Weckert & Hümmer, 1997).

Using a second-order Born approximation (Shen, 1986), it can be shown that the normalized interference intensity $I_{\mathbf{H}}$ in RBD geometry is given by

$$I_{\mathbf{H}}(\theta) = 1 + 2|F_{\mathbf{H-G}}/F_{\mathbf{H}}|[R_{\mathbf{G}}(\theta)]^{1/2}\cos[\delta + \nu_{\mathbf{G}}(\theta)], \qquad (1)$$

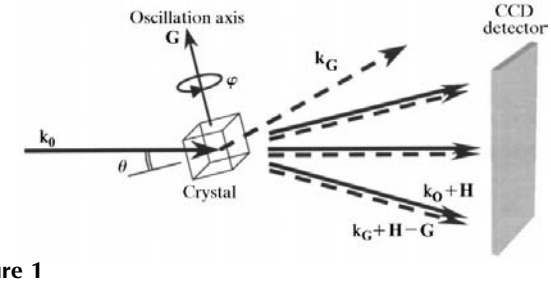
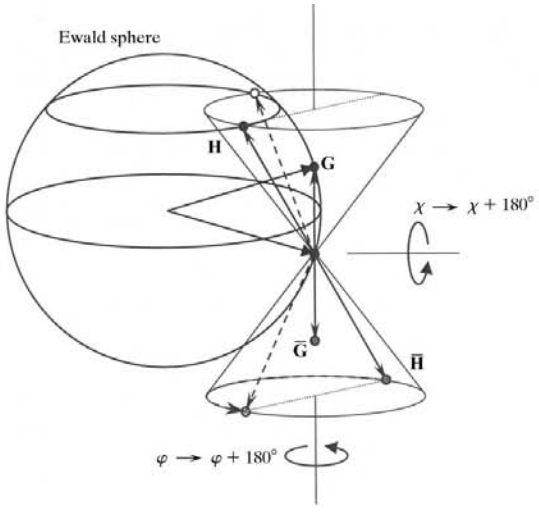


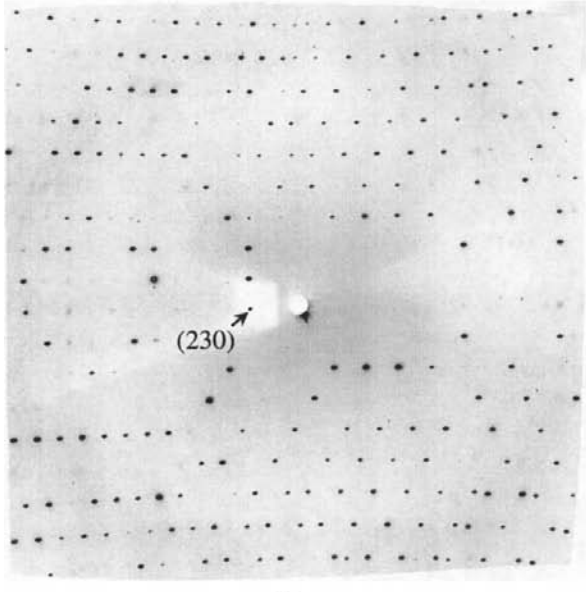
Figure 1
Schematic of the reference-beam diffraction (RBD) technique that incorporates the three-beam measurements into a standard oscillation camera set-up. Two sets of diffraction patterns, one produced by the incident beam $\mathbf{k_0}$ and the other by the Bragg beam $\mathbf{k_G}$ of the reference reflection **G**, interfere with each other and generate a phase-sensitive diffraction image on an area detector. Complete RBD interference profiles are recorded as a series of multiple frames as a function of rocking angle θ corresponding to different positions on the **G** reflection rocking curve.

where $R_{\mathbf{G}}(\theta)$ is the reflectivity, $\nu_{\mathbf{G}}(\theta)$ is the phase shift given in dynamical theory for reference reflection **G**, $F_{\mathbf{H}}$ and $F_{\mathbf{H-G}}$ are the structure factors for reflection **H** and for coupling reflection **H**−**G**, respectively, and $\delta = \alpha_{\mathbf{H-G}} + \alpha_{\mathbf{G}} - \alpha_{\mathbf{H}}$ is the triplet phase of the reflections involved (Shen, 1998). A more rigorous distorted-wave theory (Shen, 1999*b*) can be used to derive equation (1), which also takes into account the phase-insensitive kinematic peak intensity at the center of the **G** reflection rocking curve. For practical purposes, the reflectivity $R_{\mathbf{G}}(\theta)$ can be substituted by a Lorentzian and the dynamical phase shift $\nu_{\mathbf{G}}(\theta)$ can be approximated by a hyperbolic tangent function, as demonstrated by Shen (1999*a*). We would like to note that there was an error in our previous publications (Shen, 1998, 1999*a*), where the factor 2 in equation (1) was missing because $[R_{\mathbf{G}}(\theta)]^{1/2}\exp(i\nu_{\mathbf{G}})/w$, with w being the Darwin width, should be equal to $1/(2\Delta\theta)$ instead of $1/\Delta\theta$ as in Shen's original paper (1998). This error does not, however, affect the data analyses in practice since a fitting parameter was used for the coefficient in front of the interference term in equation (1). The principle of using RBD for enantiomorph determination relies on the fact that equation (1) contains a term involving $\sin\delta \sin\nu_{\mathbf{G}}$, which depends on the sign of δ and is most sensitive at the center of the **G** rocking curve where $\nu_{\mathbf{G}}$ is close to $\pi/2$.

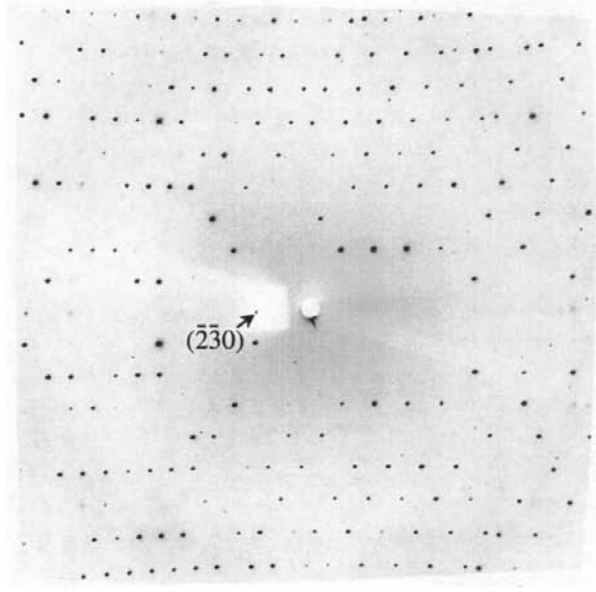
To demonstrate this procedure, we have performed a reference-beam X-ray diffraction experiment on a tetragonal lysozyme crystal (unit-cell dimensions $a = b = 78.54$, $c = 37.77$ Å) at the C line of the Cornell High Energy Synchrotron Source (CHESS), using unfocused 1.28 Å incident bent-magnet radiation. The lysozyme crystal used in the experiment was roughly 0.2 mm in size and was mounted in a sealed glass capillary. A standard four-circle diffractometer was used to align **G** = (230) as the reference reflection, with a structure-factor magnitude $|F_{\mathbf{G}}| = 2310$. A 1K × 1K charge-coupled device (CCD) was used to collect the oscillation data. As illustrated in Fig. 2(*a*), the inverse-beam geometry with $(\bar{2}\bar{3}0)$ as the new reference beam was achieved by rotating φ to $\varphi + 180^\circ$ and χ to $\chi + 180^\circ$ simultaneously. The two related diffraction images for the same oscillation range $\Delta\varphi$ were a mirror image of one another, as shown in Figs. 2(*b*), (*c*). Automatic indexing using standard crystallographic routines such as *DENZO* (Otwinowski & Minor, 1997) confirmed that all reflections recorded in the two frames are related by Friedel pairs.



(*a*)



(*b*)



(*c*)

Figure 2
(*a*) Reference-beam Friedel-pair measurements using an inverse-beam geometry on a standard four-circle diffractometer. (*b*) and (*c*) Two typical reference-beam diffraction images related by inverse-beam measurements, which are mirror images of one another. The rectangular shaped shadow around the reference reflection (230) or $(\bar{2}\bar{3}0)$ is the result of an attenuator that is used to prevent intensity saturation and allows a simultaneous recording of the reference reflection rocking curve.

A series of 25 such image pairs were collected as a function of the **G** reflection rocking angle θ at different positions on the **G** rocking curve. By using an appropriate attenuation foil (shown as a rectangular white shadow in each image in Fig. 2), the intensity of the reference reflection **G** = (230) or **G** = $(\bar{2}\bar{3}0)$ is faithfully recorded on the corresponding data frame, which ensures that the center of the reference reflection rocking curve is well defined for data analysis. All Bragg reflections recorded on each CCD frame were integrated using *DENZO* and automatically scaled using *SCALEPACK* (Collaborative Computational Project, Number 4, 1994), and the end product after this automated analysis was a series of integrated intensities $I_{\mathbf{H}}(\theta)$ as a function of θ and their Friedel pairs $I_{\bar{\mathbf{H}}}(\theta)$.

Two such inversion-symmetry-related intensity profiles are shown in Figs. 3(*a*), (*b*), for the $(3\bar{2}4)/(230)$ and the $(\bar{3}2\bar{4})/(\bar{2}\bar{3}0)$ reflections, along with a rocking curve of (230) in Fig. 3(*c*). The solid curves in Figs. 3(*a*), (*b*) are the automatic best fits to equation (1), yielding triplet phase values of $\delta = 77^\circ$ for the $(\bar{3}2\bar{4})$ and $\delta = -116^\circ$ for the $(3\bar{2}4)$, respectively. The errors of the fits arise mostly from random counting statistics, but it is possible that theoretical approximations used in (1) and the effect of other nearby simultaneous reflections may also contribute to the errors. For comparison, the two possible phases calculated using Protein Data Bank entry 193L (Vaney *et al.*, 1995) are +91 or -91° for the $(\bar{3}2\bar{4})$ and -91 or $+91^\circ$ for the $(3\bar{2}4)$, depending on whether the enantiomorph is $P4_32_12$ or $P4_12_12$. Even though the best-fit values are about 20° away from $\pm 91^\circ$ owing to measurement errors, it is still obvious and unambiguous to conclude from these RBD measured triplet phases that the correct enantiomorph has to be $P4_32_12$.

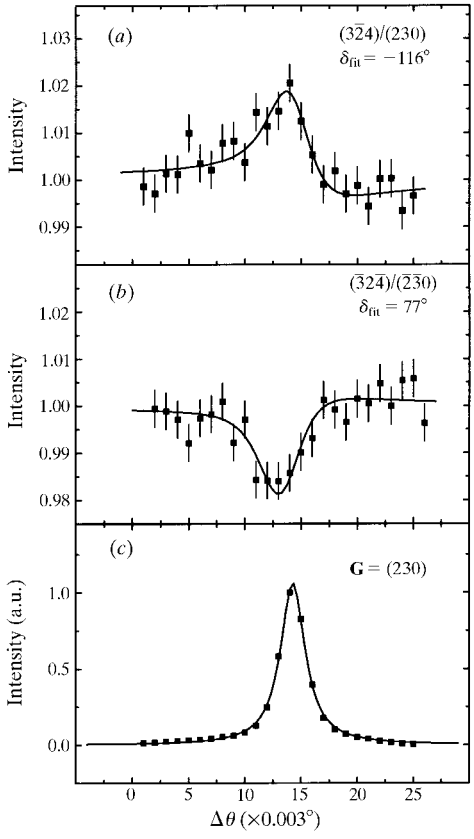


Figure 3
Measured diffraction profiles of a reference-beam Friedel pair, $(3\bar{2}4)/(230)$ in (*a*) and $(\bar{3}2\bar{4})/(\bar{2}\bar{3}0)$ in (*b*), with solid curves being the best fits using equation (1), yielding the listed triplet phase values. The rocking curve of the reference reflection (230) is shown in (*c*), which was recorded simultaneously on the same series of frames as those in (*a*).

3. Discussion and conclusions

In principle, once an oscillation diffraction pattern is unambiguously indexed (Colella, 1995), it is not necessary to go through the inverse-beam geometry to determine the enantiomorph since all the phase information is already contained in the reference-beam interference profile (1). In practice, however, it is essential to measure the Friedel pairs directly using the inverse-beam setting so that phase-insensitive intensities that arise from the kinematic *Umweganregung* or *Aufhellung* effect (Renninger, 1937; Moon & Shull, 1964) can be eliminated from the phase measurements. This procedure has already been proposed and used in the conventional ψ-scanning method for multibeam experiments (Weckert & Hümmer, 1997).

Although a single pair of inverse RBD profiles is enough for an enantiomorph determination of a given crystal, as illustrated above, the data-collection efficiency of the RBD method allows simultaneous measurements of many three-beam combinations with their triplet phases close to $\pm 90^\circ$. This data redundancy would increase the reliability of the enantiomorphic phases determined in a RBD experiment. The similarity between the RBD data collection and ordinary crystallographic data collection may also make it possible to include the RBD Friedel-pair measurements in standard techniques such as MAD when anomalous signals are relatively weak. It would also be interesting to include the enantiomorph information obtained by RBD in a unified direct-method approach (Weeks *et al.*, 1998) to extend the applicability of the *ab initio* phasing methods into a regime of solving larger macromolecular crystal structures.

In conclusion, the RBD method in inverse-beam geometry can be used to experimentally determine both the absolute values and the signs of the triplet phases of a large number of Bragg reflections recorded in a standard oscillating crystal set-up. For a triplet phase close to $\pm 90^\circ$, the measured RBD interference profiles are particularly sensitive to the sign of the triplet phase and this information can be used to distinguish the enantiomorph of a specific noncentrosymmetric space group.

The authors would like to thank Park Doing, Jim Lauippa, Marian Szebenyi, Chris Heaton and Ken Finkelstein at CHESS for their assistance during various stages of the experiment. This work is supported by the National Science Foundation through CHESS under Award No. DMR 97-13424.

References

Chang, S. L. (1982). *Phys. Rev. Lett.* **48**, 163–166.
Chang, S. L., Chao, C. H., Huang, Y. S., Jean, Y. C., Sheu, H. S., Liang, F. J., Chien, H. C., Chen, C. K. & Yuan, H. S. (1999). *Acta Cryst.* A**55**, 933–938.
Chang, S. L., King, H. E. Jr, Huang, M.-T. & Gao, Y. (1991). *Phys. Rev. Lett.* **67**, 3113–3116.
Chapman, L. D., Yoder, D. R. & Colella, R. (1981). *Phys. Rev. Lett.* **46**, 1578–1581.
Colella, R. (1974). *Acta Cryst.* A**30**, 413–423.
Colella, R. (1995). *Acta Cryst.* A**51**, 438–440.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Hart, M. & Lang, A. R. (1961). *Phys. Rev. Lett.* **7**, 120–121.
Hendrickson, W. (1991). *Science*, **254**, 51–58.
Juretschke, H. J. (1982). *Phys. Rev. Lett.* **48**, 1487–1489.
Moon, R. M. & Shull, C. G. (1964). *Acta Cryst.* **17**, 805–812.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Post, B. (1977). *Phys. Rev. Lett.* **39**, 760–763.
Renninger, M. (1937). *Z. Phys.* **106**, 141–176.
Shen, Q. (1986). *Acta Cryst.* A**42**, 525–533.
Shen, Q. (1998). *Phys. Rev. Lett.* **80**, 3268–3271.
Shen, Q. (1999*a*). *Phys. Rev. B*, **59**, 11109–11112.
Shen, Q. (1999*b*). *Phys. Rev. Lett.* **83**, 4784–4787.
Shen, Q. & Colella, R. (1987). *Nature (London)*, **329**, 232–233.
Shen, Q. & Finkelstein, K. D. (1990). *Phys. Rev. Lett.* **65**, 3337–3340.
Shen, Q., Kycia, S. & Dobrianov, I. (1999). XVIIIth IUCr Congress, Glasgow, Scotland, Abstract M07.OA.005.
Tischler, J. Z. & Batterman, B. W. (1986). *Acta Cryst.* A**42**, 510–514.
Vaney, M. C., Maignan, S., Ries-Kautt, M. & Ducruix, A. (1995). Deposited with Protein Data Bank, PDB ID = 193L.
Weckert, E. & Hümmer, K. (1997). *Acta Cryst.* A**53**, 108–143.
Weeks, C. M., Miller, R. & Hauptman, H. A. (1998). *Direct Methods for Solving Macromolecular Structures*, edited by S. Fortier, pp. 463–468. Dordrecht: Kluwer Academic Publishers.