ESRF	**Experiment title:** Experimental Study of the Anisotropy of Anomalous Scatterers of Importance in Macromolecular Crystallography	**Experiment number:** 01–02–281
Beamline: BM01A	**Date of experiment:** from: 29–nov-00 to: 4–dec–00	**Date of report:** 26–feb–01
Shifts: 18	**Local contact(s):** Phil Pattison, Silvia Capelli	*Received at ESRF:*

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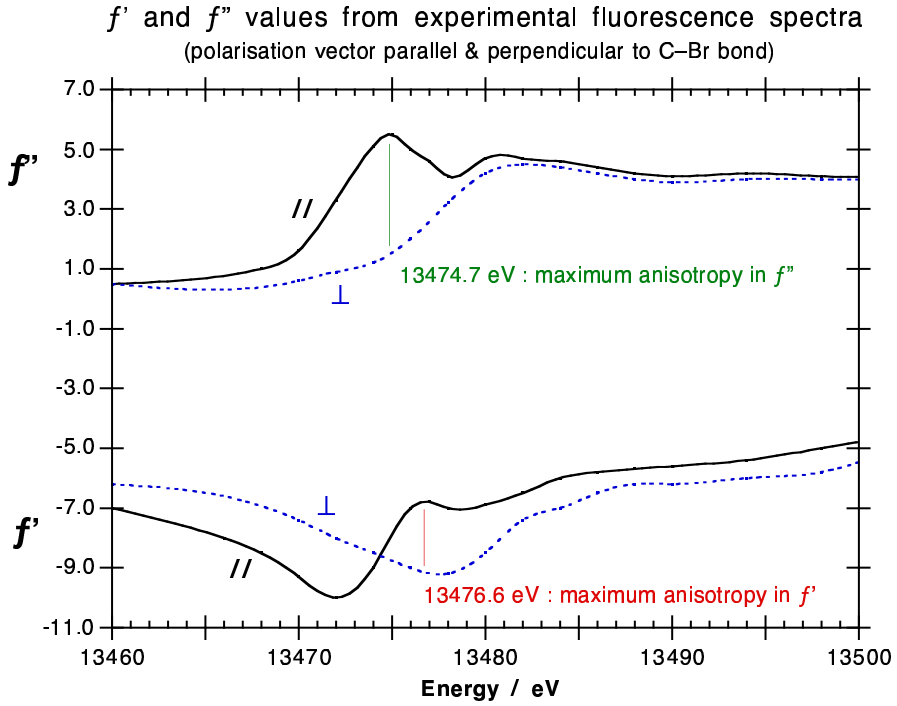
Report:

First experiments for this study were conducted on the Swiss-Norwegian Beamline at the ESRF during a 6-day beamtime period from November 29th to December 4th. One extra-day of beamtime on November 27th was used to screen samples and perform preliminary X-ray characterisations of crystals.

The study focused exclusively on 5-Bromo-Uridine, a molecule that is of importance in macromolecular crystallography since it can be naturally incorporated into nucleic acids and, as such, it is frequently used to solve DNA structures by anomalous scattering methods at the Br *K*-edge.

(1) Preliminary characterisations : A number of crystals had been grown and the most promising samples had been tested on a rotating anode and *KUMA* diffractometer at the Institute of Crystallography of the University of Lausanne. However, with the very parallel beam at the *SNBL*, all of these crystals turned out to be somewhat mosaic, showing shouldered and/or elongated scan profiles for high-angle reflections. Further screening of crystals on site was therefore necessary and this was done during the one day of extra-beamtime. Eventually, a crystal of size 400 × 150 × 100 μm^3 exhibiting symmetric and very narrow reflection profiles was selected. A full dataset at a wavelength of 0.7 Å was recorded with the single-axis goniometer and the *MAR* area-detector (image plate) set-up. In all regions of reciprocal space, the crystal showed single reflection profiles. All subsequent experiments were conducted on this crystal.

(2) Fluorescence spectra on the sample were recorded at energies close to the Br *K*-edge with the crystal in two orientations where the polarisation vector of the X-ray beam was respectively parallel and perpendicular to the plane containing the C–Br bonds. Since it is essentially this bond that breaks the spherical symmetry of the Br atoms, the modulation of fluorescence spectra due to anisotropy was expected to be largest for these two orientations and this is indeed what is observed. The fluorescence spectra were converted to *f*" plots by application of the optical theorem and to *f*' plots by application of Kramers-Kronig transformation. Results are shown in the figure below.

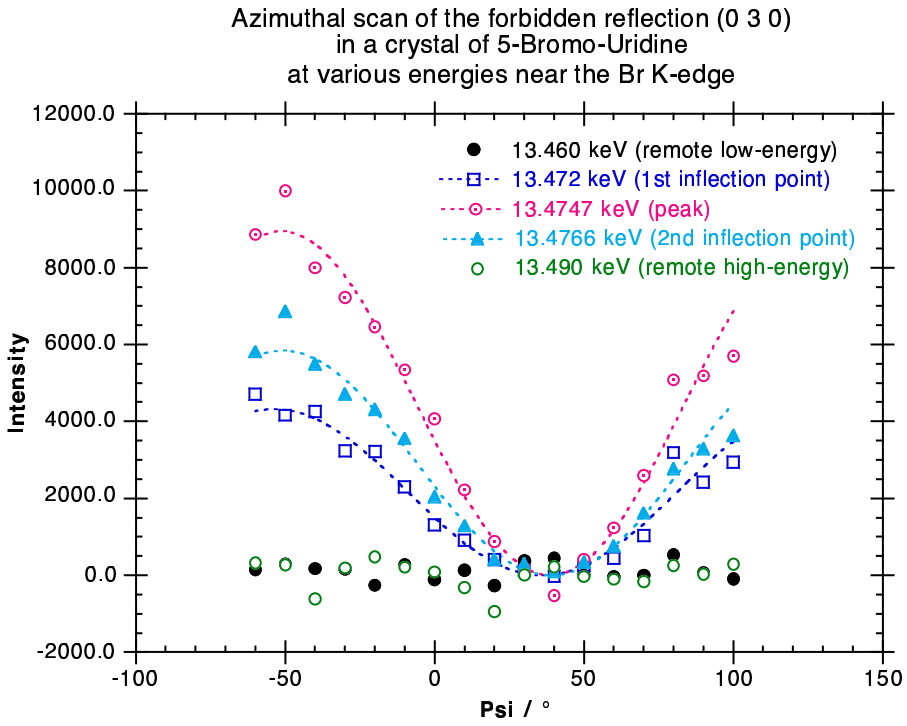


(3) Complete data collections : Based on the fluorescence spectra, two energies were selected at 13475 eV and 13477 eV where the anisotropies in respectively *f*" and *f*' are largest. A third low-energy remote wavelength was selected at 13460 eV where anomalous scattering is minimal. At these three wavelengths, complete spheres of data up to a maximum resolution of 1.05 Å were collected on the *KUMA* diffractometer. With the crystals belonging to the Laue group 2/m, every unique reflection has three symmetry-related mates that are no longer equivalent due to anomalous scattering (which breaks Friedel equivalence) and to anisotropy (which breaks the remaining symmetry). By measuring complete spheres, the four Laue-related observations of every unique reflection can therefore be compared to each other. The observed intensity differences can then be used to refine anisotropy tensors of the Br atoms. The third, remote wavelength serves as a standard for comparisons and will also be useful for scaling the various datasets against each other. Each of these three data collections took about 24-28 h for the measurement of some 2200 reflections. Some technical problems occurred : after correction by monitor-counts (incident beam intensity), the reference reflections display a small but gradual intensity decay in between beam-refills. However, at refill points, the corrected intensities of these reflections show discontinuities. This behaviour is probably due to beam instabilities in 16-bunch mode.

(4) Collection of smaller datasets with Ψ-modulation : A list of some 50 unique low-resolution reflections that are predicted to be most sensitive to anisotropy and to orientational variations was computed. The

diffractometer software was programmed to collect each of these reflections + its three Laue symmetry-related mates at Ψ-angles of –36°, –12°, +12° and +36°. Collection of one such dataset, which comprises about 700-800 measurements, took about 12 h. These data were collected at 6 different energies across the Br *K*-edge : 13468, 13472, 13474, 13476, 13478 & 13480 eV. Care was taken to start and complete each data collection in between two beam refills to avoid the above-mentioned problems. Full use was made of the possibilities of the *KUMA*-diffractometer to collect data at various Ψ-angles, but also to use the bisecting mode for measuring Friedel-related reflections in 'anti-positions'. In this geometry, the absorption of X-rays by the crystal is essentially the same for the two Friedel-measurements. Thus, absorption errors will cancel out when forming anomalous differences $[F_+(\Psi) - F_-(\Psi)]$. An absorption-error-free refinement of anisotropy tensors can therefore be conducted against these intensity (or amplitude) differences.

(5) Ψ-scans on forbidden reflections : Anisotropy of anomalous scattering breaks the crystal symmetry and forbidden reflections may therefore have non-zero intensities. The intensities of these reflections are predicted to display a marked variation with azimuthal angle. Bromo-Uridine crystals belong to the space-group $P2_1$ and the only forbidden reflections are those of type (0 *k* 0) with odd *k*. Full Ψ-scans were performed on reflections (0 7 0), (0 5 0), (0 3 0), (0 1 0) and (0 –7 0), at an energy of 13475 eV. All scans show important intensity variations as a function of azimuthal angle. For reflection (0 3 0), the scans were repeated at 3 different wavelengths across the edge and at 2 remote wavelengths respectively on the high- and low-energy side of the edge. Results are shown in the figure below. Only at edge wavelengths, were there is significant anisotropy does this forbidden reflection have non-zero intensity. The intensity variation as a function of Ψ displays the expected behaviour. Such Ψ-scans on forbidden reflections can therefore be useful to detect anisotropy of anomalous scattering and, more important, to select the wavelengths were these effects are most marked. We suggest that this might be a viable alternative to recording fluorescence spectra, especially in cases where there are many anomalous sites at different orientations (which is often the case in macromolecular crystals) which, by macroscopic averaging, would give rise to isotropic fluorescence spectra.



(6) Further experiments : A reference dataset of the same crystal was later collected at the *KUMA* diffractometer at the Institute of Crystallography in Lausanne with Mo $K\alpha$ radiation. At the same time the crystal faces were indexed. This will allow to carry out analytical absorption corrections. A further data collection at higher resolution is planned.

(7) Processing of the diffraction data is currently in progress. Because of the significant absorption of X-rays at wavelengths close to the Br-edge, correction models have to be chosen very carefully. A number of such correction models, both analytical and empirical, are currently tested. Also, because of the discontinuities in the intensity of the reference reflections, the 'large' datasets have to be split up into several segments that must be processed independently and then scaled to each other. Once the processing of the data is accomplished, anisotropy tensors will be refined, using specially developed software. Finally, for the 'large' datasets, an attempt will be made to exploit the anisotropy for determining the structure factor phases.