



	Experiment title: Experimental study of the anisotropy of anomalous scatterers of importance in protein crystallography	Experiment number: LS1842
Beamline: BM01A	Date of experiment: from: 13 june 2001 to: 21 june 2001	Date of report: 27-feb-01 <i>Received at ESRF:</i>
Shifts: 21	Local contact(s): S. CAPELLI & P. PATTISON	

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Report:

The measurements conducted during this beamtime slot are part of an ongoing project aimed at characterising the anisotropy of resonant anomalous scattering in compounds of biological interest. Previous experiments were conducted on a Br-containing small molecule compound [ESRF experiment report 01-02-281]. They allowed us to establish various experimental protocols for characterizing this phenomenon. In the present round of experiments we did, for the first time, apply these protocols to study the anisotropy of anomalous scattering in a macromolecular crystal. The samples used were selenium-containing crystals of the protein human Aldose reductase (molecular weight: 36 kg/mol) [Lamour *et al.*, (1999). *Acta Crystallogr.* **D55**, 721-723] which diffract to very high resolution (better than 1 Å). Five sulfur atoms per molecule were substituted by selenium atoms *via* genetic engineering. All experiments were carried out at the Se *K*-edge.

In this first round of experiments the main goal was to establish the feasibility of collecting diffraction data on a frozen macromolecular crystal on the six-circle diffractometer installed at BM01A. In addition, the strategy of detecting anisotropy of anomalous scattering with Ψ -scans was validated.

Specific outcomes of these experiments are:

- (1) Successful measurement of single reflections on a frozen macromolecular crystal with the 6-circle diffractometer and point detector. Computation and refinement of an orientation matrix for such a large unit-cell, using the standard software package installed with the diffractometer.

- (2) Recording of fluorescence spectra across the Se *K*-edge at various orientations of the crystal with respect to the direction of polarization of the incident beam (see figure 1), exhibiting the anisotropy of X-ray absorption, which is closely related (*via* the optical theorem) to the anisotropy of anomalous scattering.
- (3) *DANES* (Diffraction Anomalous Near-Edge Structure) scans of the intensity of selected reflections across the Se *K*-edge (see figure 2), exhibiting the effect of resonant scattering in the measured intensity.
- (4) Ψ -scans of selected reflections at various energies close to the absorption edge (see figure 3), exhibiting directly the anisotropy and the orientation/polarization dependence of anomalous scattering in the diffracted intensities.

These results show that the anisotropy of resonant scattering of only a few selenium atoms in a macromolecule produces sizeable modulations of both the macroscopic absorption (as measured by fluorescence) and the diffracted intensities, as the orientation of the crystal is varied with respect to the direction of polarization of the incident X-ray beam. Further measurements will be necessary to quantify these effects and to extract numerical data in the form of a tensor description for f' and f'' factors.

The anisotropy of X-ray absorption (as observed in fluorescence spectra) has been occasionally observed in macromolecular crystals during the preliminary steps in multi-wavelength experiments. Our measurements are however the first reported direct observations of anisotropy of X-ray scattering in diffracted intensities on such crystals. The main motivation of this study is the exploitation of this phenomenon for the determination of structure factor phases.

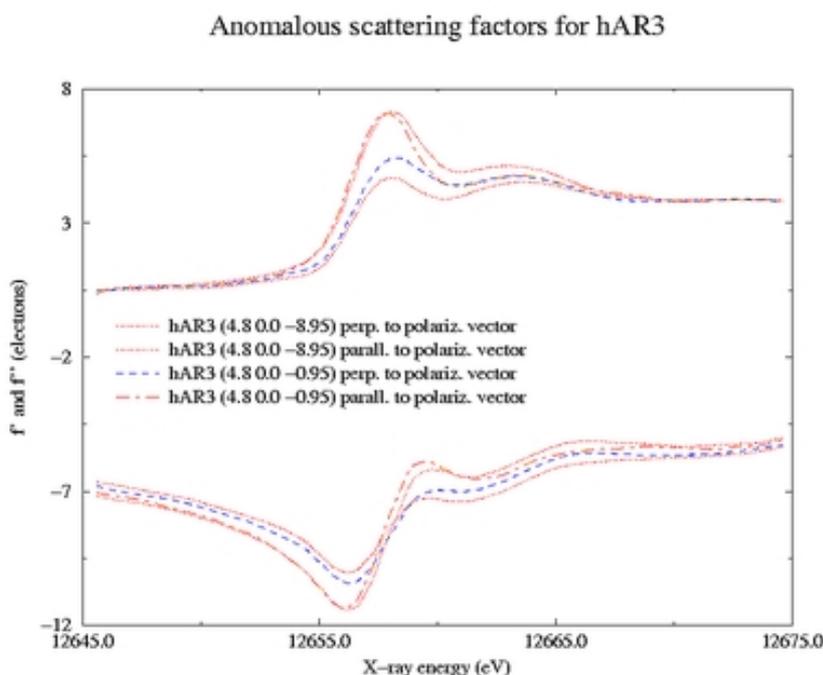


Figure 1. Scattering factors f' & f'' as obtained from fluorescence spectra. Fluorescence spectra were recorded with the crystal in four different orientations with respect to the polarization direction of the incident beam. The fluorescence intensities were converted to f'' plots by application of the optical theorem and to f' curves by Kramers-Kronig transformation.

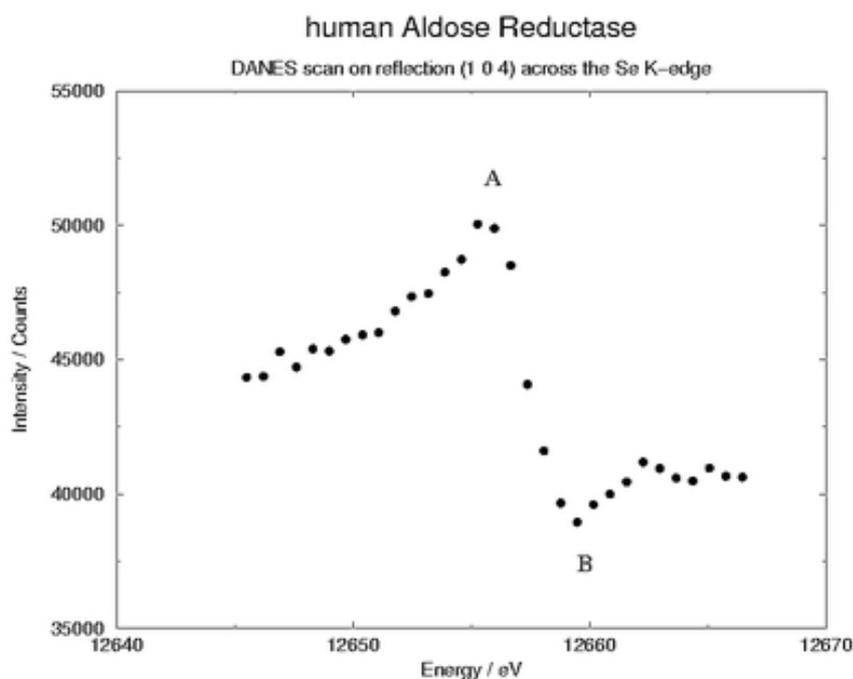


Figure 2. Near-edge scan of the diffracted intensity of reflection (1 0 4). This is a centric reflection, for which the complex structure factors of the Se and the remaining atoms align. For this particular reflection, these two structure factor contributions have opposite signs. The observed intensity variations therefore essentially reproduce the modulations of $-f''$ for the Se atoms. These modulations display two extrema (A and B).

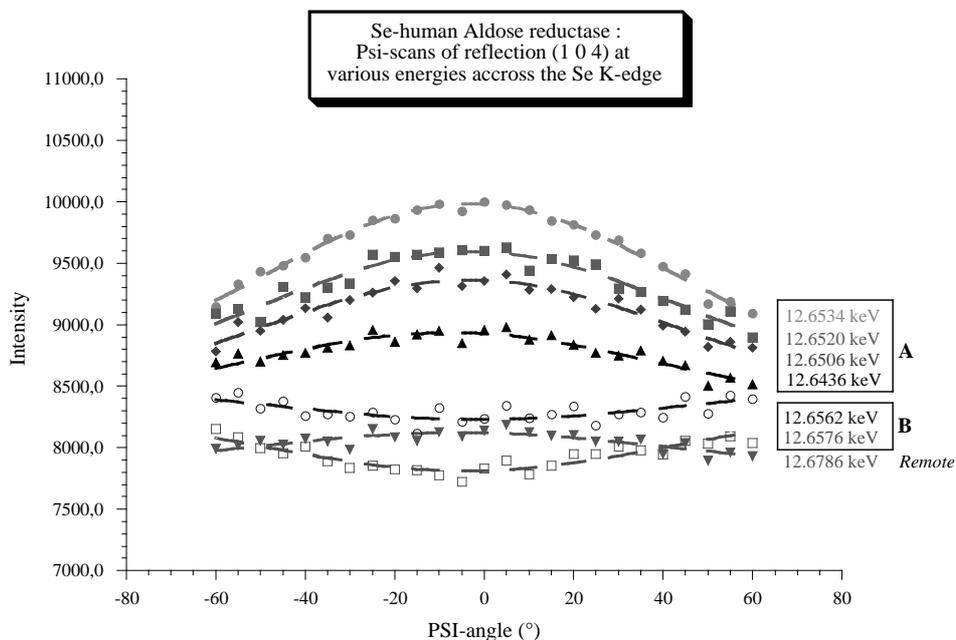


Figure 3. Ψ -scans of reflection (1 0 4) at various energies across the Se K -edge. The intensity variations observed in these scans can be directly related to the anisotropy of f'' . Note that at the two wavelengths where f'' as a function of energy goes through extrema (A and B), the Ψ -plots display opposite curvatures. This feature can only be explained by the anisotropy of the scattering factor f'' (and not by absorption effects).