4 Data Collection

For native and derivative data collection, a crystal was mounted in a glass capillary (W. Müller, Berlin, Germany) with a diameter of 0.7 mm and a wall–thickness of 10 μm. At both ends of the capillary, some mother liquor was added in order to prevent the drying out of the crystal during data collection and the capillary was sealed with hot wax. Native and derivative crystals were soaked in mother liquor for two hours before mounting and data collection in order to improve isomorphism. X–ray diffraction data were collected to 4.5 Å resolution on the Mar Research imaging plate detector and beyond 2 Å at the Photon Factory.

4.1 High Resolution Data Collection

High resolution X–ray diffraction data were collected using the synchrotron facilities at the National Laboratory for High Energy Physics (KEK, Photon Factory) in Tsukuba, Japan. The data were obtained on beamline station 6A2 using a screenless Weissenberg camera (Sakabe, 1983; Sakabe, 1991). The Photon Factory is based on a 2.5–GeV positron storage ring, with a beam current of 250 to 300 mA. Positrons or electrons can be injected into the storage ring from a 2.5–GeV linear accelerator. The data collection facility uses a 4–mrad white beam exiting through a horizontal aperture and vertically focused by a 1 m curved fused quartz mirror both to increase the brightness and to cut off higher harmonics. The monochromator is a triangularly shaped and asymmetrically cut Si(III) crystal which is bent to provide horizontal focusing. A 0.1 mm diameter collimator was used directly in front of the sample. The wavelength was 1 Å, and a 430 mm diameter Weissenberg camera was used. To reduce absorption and scattering of the diffracted beam by air, a helium chamber was inserted between the crystal, detector and collimator.

X–ray data were recorded using Fuji imaging plates which are coated with a storage phosphor ($BaFBr : Eu^{2+}$). When X–ray photons strike this surface the europium is ionised, allowing an X–ray image to be stored on the plate. The plate can be read by laser stimulated luminescence, and the X–ray image can be erased by an intense flash of light. The imaging plate is then irradiated with visible radiation which stimulates the Eu^{3+} to revert to Eu^{2+}. Fluorescence is emitted during this process and the intensity of the stimulated fluorescence is proportional to the X–ray intensity which produced the original $Eu^{2+} \rightarrow Eu^{3+}$ transition. A 20 cm by 40 cm imaging plate with a dynamic range of intensity of 5 orders of magnitude was used. The imaging plates were scanned on a Fujix BA100 flat–bed type densitometer (0.1 mm raster) driven by a NEC–PC9800 computer (Sakabe, 1991).

Two crystals of size 0.5 mm x 0.5 mm x 1.0 mm were used. Each crystal was first aligned by eye and then by taking 2^o oscillation photographs on polaroid film with a crystal to film distance of 150 mm and an exposure time of 6 s. Once the crystal was aligned, the beam stop was temporarily removed in order to allow the direct beam to mark three fiducial points on the imaging plate on a line along the rotation axis. The fiducial marks were used to determine the initial matrix Q, which is a 2 x 3 detector orientation matrix composed of a two–dimensional rotation matrix and a translation vector relating the detector and scanner coordinates (Rossmann, 1979). 63^o of data collected about the crystallographic c–axis were obtained as 5^o oscillations with angular overlap of 0.5^o, a coupling constant between crystal rotation and plate translation C of 2.5^o mm^{-1} and a rotation speed of 2^o s^{-1}. For the blind region, 71.7^o of data collected about the *110*–axis were obtained as above. The crystals were translated by 0.1 mm along the rotation axis after every four exposures to expose fresh non–irradiated portions of the crystal to the beam. Figure 5 shows a typical Weissenberg diffraction pattern (recorded on an imaging plate) of a native Neu5Ac lyase crystal rotated about the c–axis. Several heavy–atom derivative and native data sets were collected at the Photon Factory. All

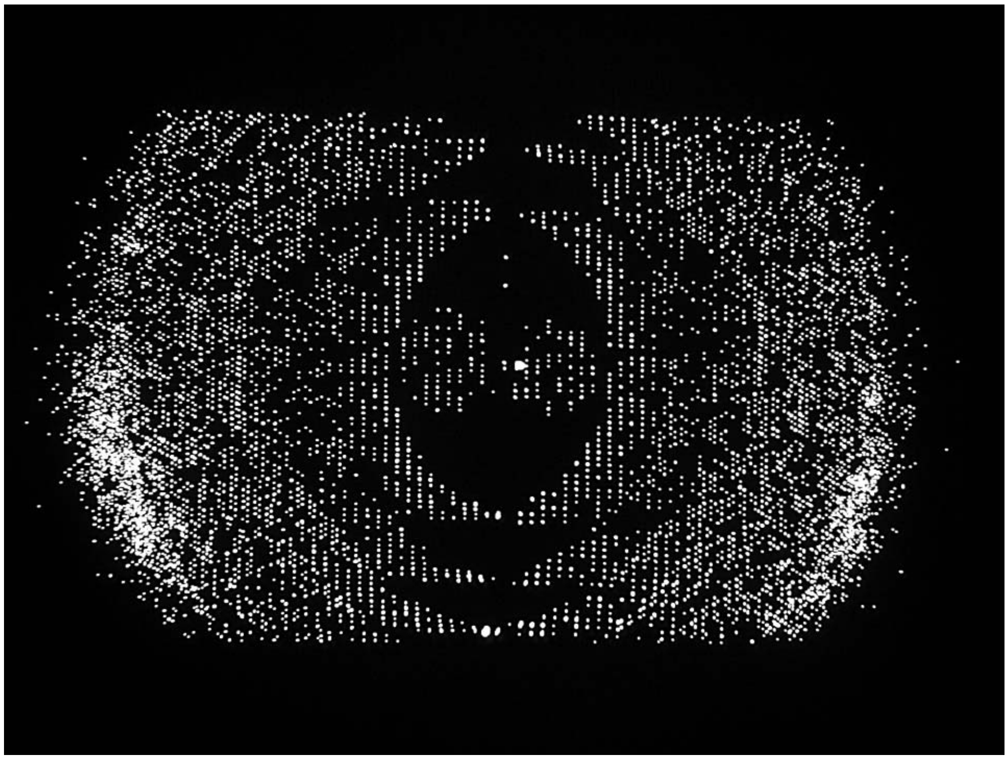

Figure 5: Diffraction pattern of a native Neu5Ac lyase crystal taken on a screenless Weissenberg camera rotating about the c–axis (vertical in this Figure). The pattern shows the diffraction in reciprocal lattice layers, with the partial reflections at the ends of each layer. The native crystals diffract beyond 2 Å resolution.

heavy–atom derivative data sets showed non–isomorphism with respect to the native data set. Efforts were therefore made to collect heavy–atom derivative data sets at low resolution under conditions very similar to those of the native data collection.

4.2 Rotating Anode Native and Derivative Data

A native Neu5Ac lyase crystal was soaked for 46 hours in mother liquor containing 1 mM diμiodobis (ethylenediamine) diplatinum (II) nitrate (PIP; purchased from Strem Chemical Company, Newport MA, U.S.A.). This crystal was then backsoaked in mother liquor over 2 hours in order to avoid excess heavy–atom compound accumulation in the solvent regions of the crystal and to improve isomorphism.

Native and derivative X–ray diffraction data sets were collected to 4.5 Å resolution on a Mar Research 18 cm diameter imaging plate detector controlled by a Vaxstation 3000. CuK_α radiation (with a weighted average wavelength of 1.5418 Å) was produced by an Elliot GX20 rotating anode generator operating at 40 kV and 45 mA with a graphite monochromator. The electrons were focused with a 200 μm focusing cup and the beam was collimated using a collimator of 0.3 mm diameter. X–ray intensities were recorded on Fuji imaging plates like the Photon Factory data. The 180 mm diameter scanner is divided into 1200 pixels x 1200 pixels with a pixel size of 150 μm x 150 μm. The scanned images are erased by illumination with intense visible light. Data to 4.5 Å resolution were collected for 60^o rotation about the c–axis in 2.5^o steps (10 oscillations per step) with an exposure time of 9000 seconds, and a crystal to imaging plate distance of 250 mm.