3 Space Group Determination and Crystal Characterisation

The Neu5Ac lyase crystals were characterised by small–angle screenless precession photography using a precession angle μ of 2^o, 20 hours of exposure time and a crystal to film distance of 100 mm on an Enraf–Nonius precession camera. The precession method allows the recording of a plane of the reciprocal lattice on a photographic film in an undistorted manner for the zero layer in this case. On a screenless small–angle precession photograph, the circular film area of the zero–level and the annular regions of upper levels which pass through the sphere of reflection are apparent and the center of the zero–level circle corresponds to the straight–through position of the X–ray beam (Buerger, 1964).

The crystals were oriented with the *c*–axis normal to the incident X–ray beam and in order to analyse the symmetry down the *c*–axis it was necessary to mount the long axis of the crystal perpendicular to the capillary axis. For analysis of the repeating distance along the *c*–axis, a precession photograph was also taken with the *110*–axis mounted normal to the incident X–ray beam, *i.e.* with the *c*–axis along the capillary. From the repeating distances on the precession photographs, the unit cell parameters were determined as $a = b = 122.8$ Å, $c = 198.9$ Å, $\alpha = \beta = 90^o$ and $\gamma = 120^o$, suggesting a hexagonal or trigonal crystal system. The crystals have volume to mass ratio, V_m, of 2.8 Å^3 Da^{-1} based on the assumption that the crystals contain one tetramer in the asymmetric unit [estimated using the formula given by

Matthews (1968) based on the unit cell contents and the protein molecular weight]. This corresponds to a solvent content of 0.56, which is within the range of normally observed for protein crystals (Matthews, 1968).

A $\mu = 2^o$ photograph of Neu5Ac lyase is shown in Figure 4. The precession photograph in Figure 4 shows six–fold symmetry on the zero–level and three–fold symmetry on the upper levels indicating a trigonal crystal system rather than a hexagonal one. Other photographs established that only reflection with $l = 3n$ were present on the line $[0, 0, l]$. The upper–level in Figure 4 in fact shows $3m$ symmetry with the mirror planes every 60^o about c^*, along a^* and b^*. This indicates the presence of two–fold axes perpendicular to and related by the three–fold axis. The two–fold axes are perpendicular to the mirror planes. Since a^* is perpendicular to b and b^* is perpendicular to a, the two–fold axes perpendicular to the mirror planes lie along the real axes a and b. This is the case in the space groups $P3_121$ and $P3_221$. The determination of the space group and the repeating distances along the axes was also confirmed by taking 1^o oscillation photographs on the Mar Research imaging plate (data not shown).

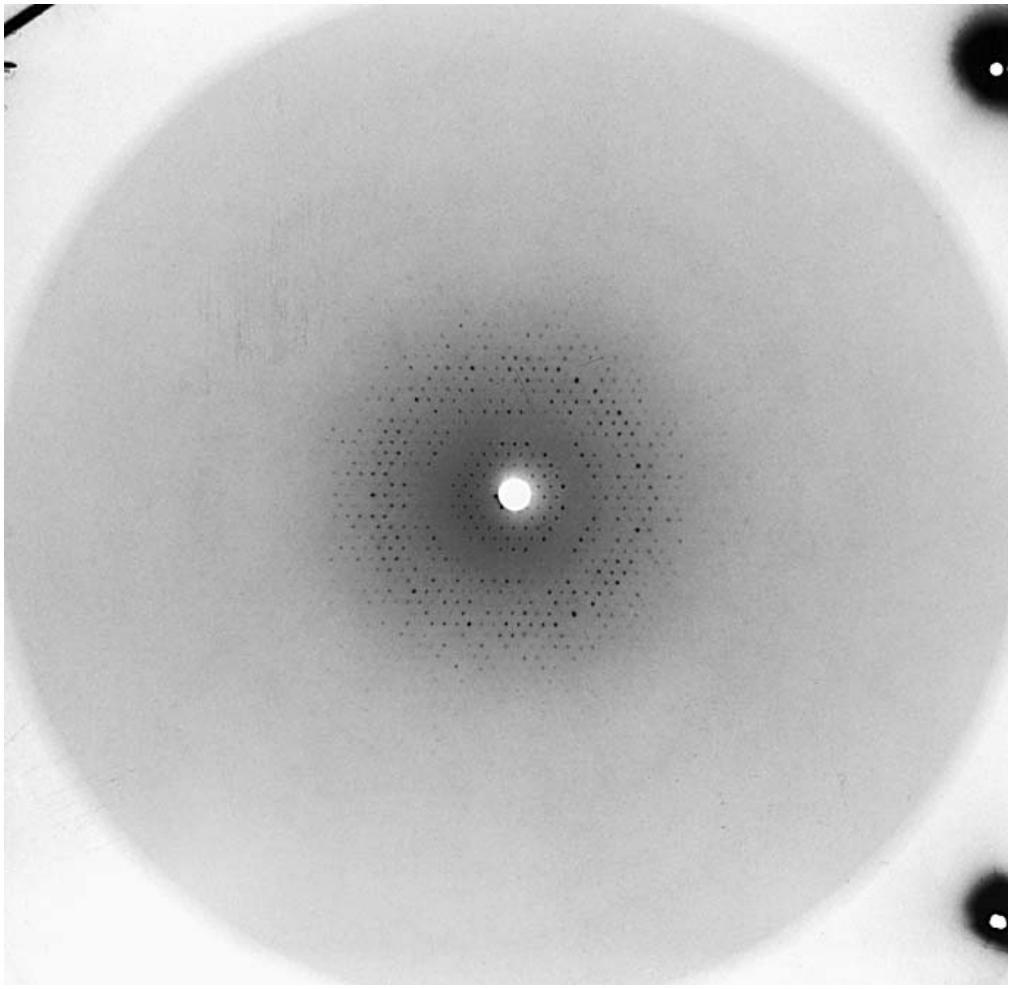

Figure 4: A screenless precession photograph of a Neu5Ac lyase crystal μ = 2^o down the c–axis. The zero–level at the film center around the direct beam shows six–fold symmetry whereas the upper levels show only three–fold symmetry.