ESRF	**Experiment title:** 'X-ray crystallographic study of the interactions between p2lras and its **GTPase** activating proteins p120GAP and neurofibromin'	**Experiment number:** LS-438
Beamline: **BL 4**	**Date of experiment** from: March, 26 **to:** March 28, 1996	**Date of report:** August 15, 1996
Shifts: **4.5**	**Local contact(s):** **Jeff** Shaw	***Received at ESRF:***

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Report:

Several crystals of the GTPase activating domain (GAP-334) of p120-GAP were tested for cryogenic data collection. Most of them showed internal disorder and were not suitable for X-ray analysis. Our GAP-334 crystals are usually thin plates their longest unit cell axis (140 Å) being along the short edge. To minimize the number of spherical overlaps the cryo-loop was bent perpendicular to the spindle axis, such that the short edge of the crystal was approximately parallel to this axis. This appeared to be important as the mosaicity increased significantly upon cryo-freezing, thus making reflection overlaps at high resolution a more serious problem at an oscillation range reasonable for the beam time available. A relatively small crystal (400x200x50 μm) diffracted to 1.6 Å after flash freezing in liquid nitrogen and was used for collection of 120 Big MAR images using a crystal detector distance of 200 mm, 0.7° oscillation range, and 12 sec exposure time. Subsequently, a Zr-attenuator (50μm) was inserted into the beam and after rotation of the crystal to its initial spindle position 90 'low' resolution Small MAR images recorded (10 oscillation range, 2 sec exposure time). Data processing with XDS (W. K.) resulted in 2 data sets of 1.6 and 2.4 Å resolution. The overall completeness of data is 96% for the high and 99% for the low resolution data set with symmetry-R-factors of 14% and 10% in the highest resolution shells.

Despite the fact that both data sets are derived from the same crystal, data merging into one set of high completeness and redundancy appeared not to be straightforward. As the completeness of both is acceptable in the range above 6 Å they are currently used independently for refinement of the GAP-334-model at 2.4 and 1.6 Å, respectively.

Since the data mentioned were obtained from an original 'test' crystal we screened for still better crystals and found one diffracting up to 1.3 Å resolution. 30 Big MAR images were collected at a 20 angle of 10° (crystal detector distance: 210 mm, oscillation range: 0.75°, exposure time: 12 see). Unfortunately, these data are incomplete (30% above 1.6 Å) for lack of beam time. Therefore and for reasons of data compatibility with the 1.6 Å set they are of limited use for refinement.

The refined GAP-334-structure, which is the first of a GTPase activating protein, should - as a homologue of neurofibromin - facilitate the solution of the phase problem in neurofibromin crystals, and in connection with the known Ras structure in complex crystals of GAP-334 or neurofibromin and Ras. We want to collect synchrotrons data of such crystals in the near future as a continuation of the experiments reported here .