ESRF	**Experiment title: Pushing The Limits Of Synchrotron Powder Diffraction: An Exploratory Study Of A Macromolecular System By PXRD.**	**Experiment number**: MI626
Beamline:	**Date of experiment**: from: 19 Sep 2002 to: 20 Sep 2002	**Date of report**: 30 Sep 2002
Shifts: 3	**Local contact(s)**: Jon Wright	*Received at ESRF:*

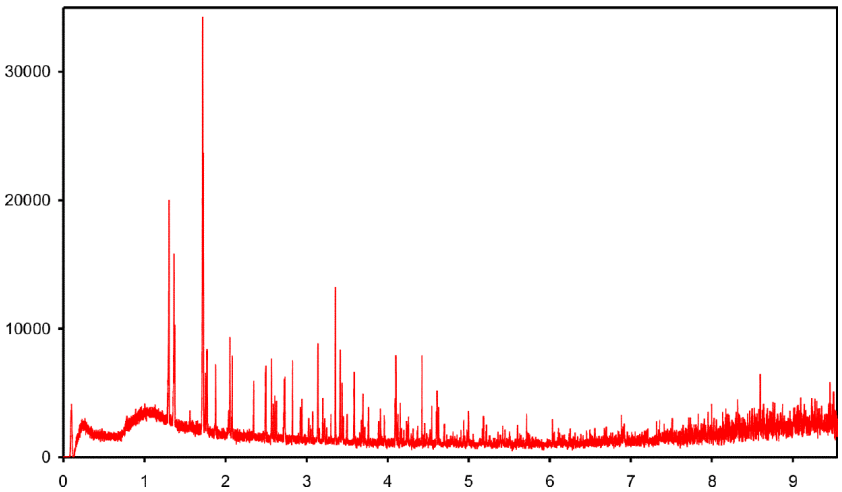
Names and affiliations of applicants (* indicates experimentalists):

*John Bond, *Jeremy Karl Cockcroft, *Ajit Basak, Paul Barnes, David Moss;

School of Crystallography, Birkbeck College, University of London, U.K.

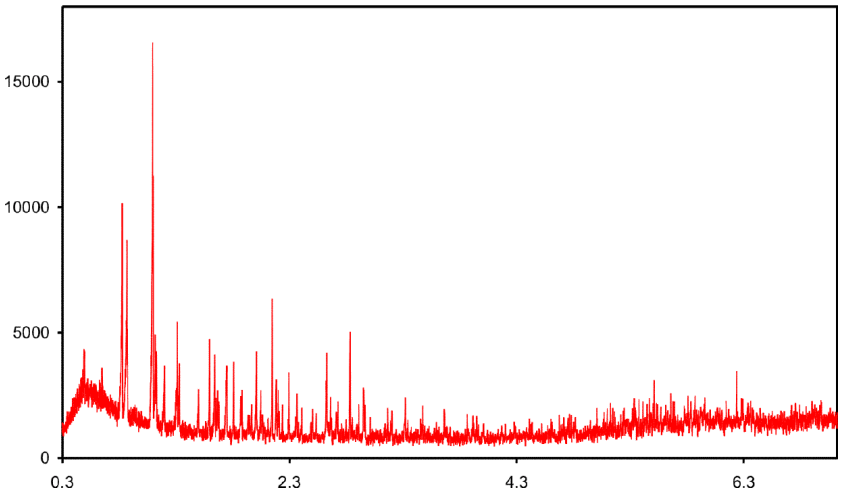
Report: The aim of this series of experiments is to explore the potential of powder diffraction for protein crystallography studies. Given the novelty of this series, the 9 shifts awarded for experiment MI626 have been divided into 3 lots of 3 shifts so that we can learn from each experiment in turn, and apply the findings to the follow-up experiments. This report describes our results from the first 3 shifts.

Our first data acquisitions on beamline ID31 were performed using a polycrystalline sample of ribonuclease A in a 0.7 mm glass capillary with an incident wavelength of 0.69 Å so as to minimise absorption and sample deterioration. As shown in the figure on the left, very high quality data could be rapidly obtained, this pattern being collected in only 2 minutes and 16 seconds. The nine detectors covered approximately the 2θ range -2º to +14.5º and the signal to noise ratio was much better than that expected based on preliminary work using our laboratory powder diffractometer.

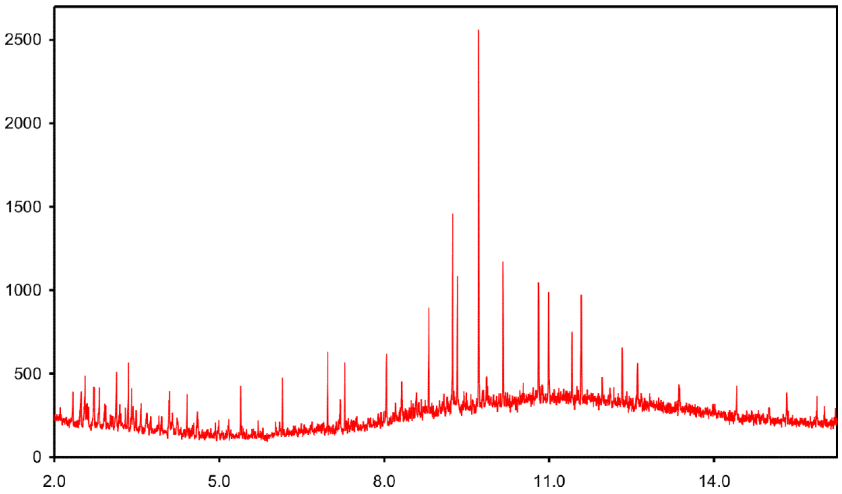


Having ascertained that we were able to collect a high-quality powder diffraction pattern of a polycrystalline protein at room temperature, our second experiment aimed to ascertain the sample lifetime in the ultra-high intensity beam provided by the undulator of ID31. The sample was scanned repeatedly and rapidly. In the full beam (100% fill mode), the sample lifetime at 0.69 Å was very short, and after approximately 10 minutes, the Bragg peaks began to reduce noticeably in intensity, and after 20 minutes, the final scan showed nothing but background. This experiment was repeated several times to check and demonstrate reproducibility. These results provided some measure of sample stability and optimum data collection times. By collecting data along the length of the capillary, and merging the repeated scans, data of better statistical quality were obtained. The height of the beam on ID31 permits sample of larger diameter, so our third set of experiments involved the use of larger bored capillaries with 1.0 mm diameter. Better results were obtained and taking this into account, even larger bored capillaries will be used during the next series of experiments in December. Finally at this wavelength, we experimented with an incident beam attenuator with regard to relative sample lifetime, and found that the latter could be significantly increased, in one case with reliable data still being collected after one hour of exposure in a 0.7 mm capillary.

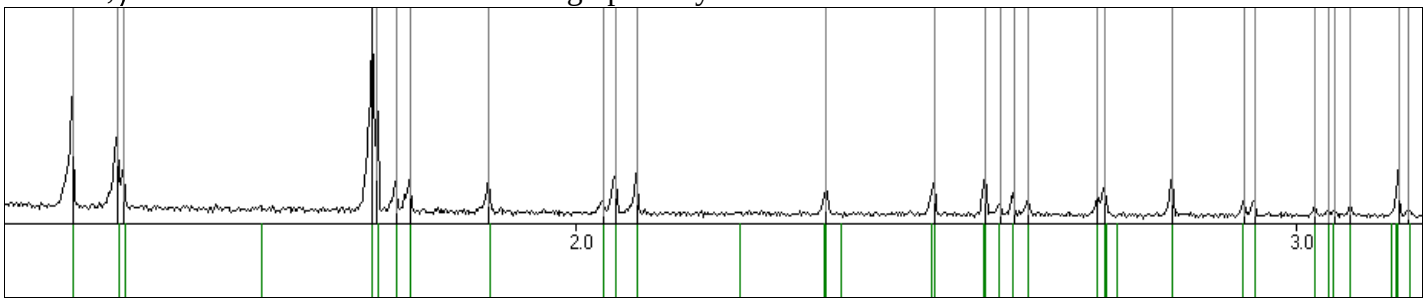
We then changed wavelength to 0.45 Å so as to investigate the effect of reduced absorption on sample lifetime. Some typical data are shown in the next figure, which depicts a single scan completed in just three minutes. With the presence of an attenuator, sample lifetime increased significantly, to nearly 90 minutes. More significant, was the discovery that the instrument resolution of the machine is such that at low angle, individual peaks were well-resolved. However, one disadvantage of such a short wavelength was the reduction in scattering power of the sample and lower peak to background count. The summed data sets obtained at this wavelength will be useful for demonstrating protein structure refinement by the Rietveld method.



Dividing the allocated 9 shifts into 3 periods has already shown to be highly beneficial since we have uncovered one unforeseen problem during this first set of experiments. Some of the salt buffer used in the crystallisation process had itself crystallised along with the ribonuclease A. Although this did not affect every sample, some extremely strong peaks in the middle of the scan range betrayed its presence. The figure on the left shows a scan collected at a wavelength of 0.69 Å. Comparison with either of the two previous figures reveals the presence of a second phase. Although the peaks appear strong, they actually correspond to a relatively small amount of contamination by weight. The denser salt was only observed in the end sections of the capillaries as a result of the sample preparation, which involved centrifugation at 1000g. Within the School of Crystallography, experiments are now being undertaken to ensure that the samples are free of this potential impurity prior to the next allocated shift.



In addition to the data collection experiments, we have made use of the acquired data to test the robustness of various software packages within the CCP14 suite. Despite knowing the unit cell a priori, we were able to index the pattern (after suitable rescaling) using the CCP14 software package CRYSFIRE. The refined parameters (CHEKCELL) provided the following monoclinic-cell values: a = 55.55 Å, b = 37.91 Å, c = 30.09 Å, β = 106.0º. The results are shown graphically below:



These unit-cell values are in good agreement with single-crystal crystallographic studies of this enzyme in this particular crystal form. The indexing revealed one problem: the peaks were so sharp that our default bin size of 0.001º proved too large, and rebinning was required.

We are now trying out various Rietveld refinements using the GSAS package and a molecular replacement method adapted for powder diffraction using PDB co-ordinates as a starting model. These results will be reported at a later stage.