ESRF	**Experiment title:** Wavelength-optimised derivative collection on proline 3-hydroxylase (Type II)	**Experiment number:** LS-906
Beamline: BM14	**Date of Experiment:** from: 8th Feb 1998 to: 10th Feb 1998	**Date of Report:** 23rd Feb 1998
Shifts: 6	**Local contact(s):** Vivian Stojanoff	*Received at ESRF:*

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Report:

At the time the experiment was carried out, we were much more confident that our phases derived from in-house derivatives were reasonable. We had a selection of crystals with various combinations of the substrates and co-factors (iron, proline and α-ketoglutarate) soaked in. We had also optimised our freezing procedure. We decided to concentrate therefore on collecting an improved native dataset and several of the soaks, using the large diameter of the Mar345 detector to give us higher resolution towards 2Å while still allowing the close spots (c axis 224Å) to be resolved.

We completed four datasets during the beamtime (Table 1).

The datasets were to 2·1–2·4Å resolution, in most cases considerably better that we have seen before (2·5Å). However, the inherent problem in our crystals, disorder associated with the long c axis, was also revealed more clearly than ever before (Fig. 1):

1. All crystals show an anisotropic fall-off in resolution perpendicular to the long c axis.
2. Spot shape can be rather poor, and there is diffuse scattering in the background.
3. Due to the long axis, an unfortunate alignment of the crystal in the cryo loop can make collection of a complete dataset extremely difficult - this affected dataset phohl.

All the data have been reduced, but evaluation of the data sets is still in progress. Careful evaluation is required to establish the degree of success of each soak and to look for the onset of conformational change. We have been unable so far to obtain crystals when all three co-factors (Fe, α-KG, proline) are present in our standard crystallization buffer, we believe that this is because the enzyme closes up on the active site when all three are bound.

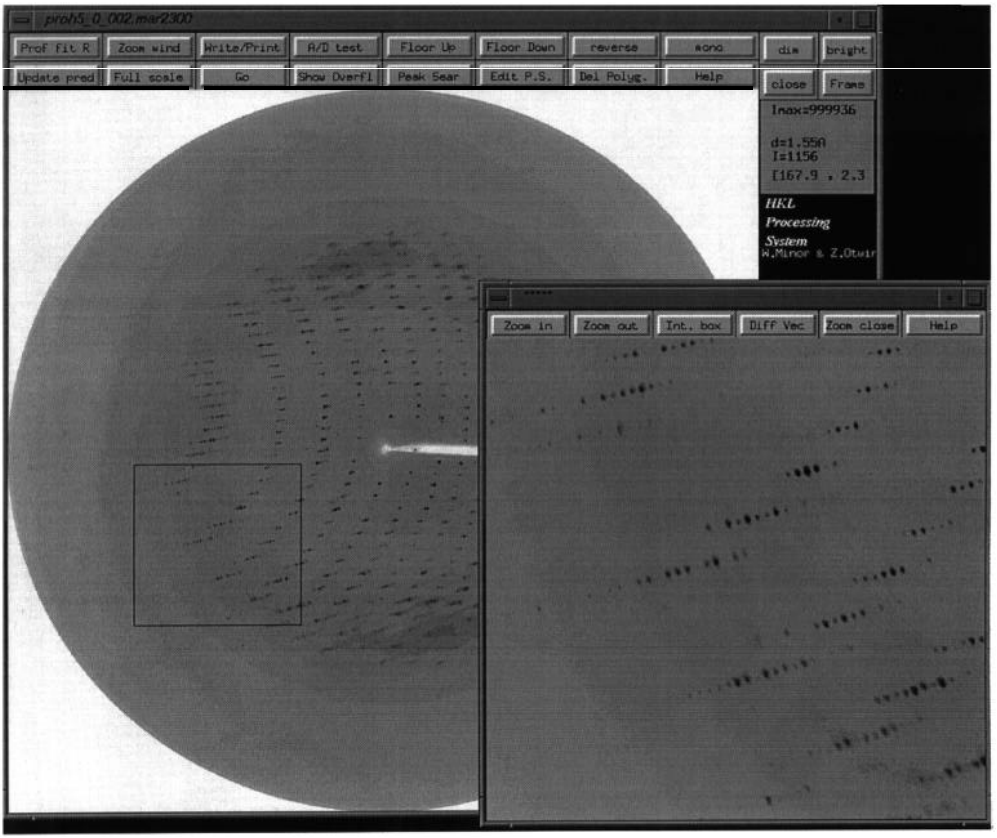

Figure 1: An image from the phoh5 dataset

Name	Crystal	frames	Comp.%	Rmeas0%	ResolÅ	$\langle I\rangle/\langle\sigma(I)\rangle$ (all)	$\langle I\rangle/\langle\sigma(I)\rangle$ (outer)
prohl	Native	83	83.0%	11.0%	2·20Å	5.3	2.1
proh2	Iron soak	59	97.4%	11.1%	2·26Å	9.6	1.8
proh5	a-KG soak	60	96.0%	11.1%	2·15Å	6.9	1.6
proh6	Proline soak	53	98.8%	10.2%	2·44Å	5.9	1.6

Table 1: Data collection statistics for proline hydroxylase