Final Report of MX386 at ID29

Several data sets have been collected during the 3 shifts at ID29 from 11th to 12th June 2005.

We have found some improvements since the last scheduled shifts, in particular we found a better system for changing wavelength and optimising the beam and we used the program DNA for data collecting, performing strategy and processing on the fly. DNA control over ProDC is quite good whenever there are no problems, but we found a little bug after the front end closed in the middle of a data collection in the middle of the night. In fact, after the BL scientist impressively understood the problem and re-opened the front end from home, we inputted DNA to start from the empty frame and its relative orientation, but ProDC re-started from frame #1 and its relative orientation; it goes without saying that we realised this after having lost the first 60 frames. So we closed both programs and reopened DNA (therefore automatically ProDC restarted) and finally collected the crystal, which had decayed in the meantime, despite having used only 10% of the beam.

The automatic crystal centring is very good, although the reaction of the camera to the zoom is a little slower than in other beamlines and the quality of the image is better in the screen outside the hutch, so we have always done was roughly centring the crystal inside the hutch and finely centring it outside.

Finally we had no problems in backing up data on our firewire disk, following the instructions on the website.

One MAD and two SAD experiments on three different needle-shaped crystals of WhiE, an aromatase/cyclase of *S. coelicolor* expressed in *E. coli* with SeMet, were performed, always after having done a scan. In both cases the three data collections were performed on sequential places along the long axis of the crystal.

In the first case the space group was C2 with the following cell dimensions: a=64.93Å, b=46.09Å, c=49.38Å, β=93.21°. Given the solvent content there is one molecule/asu, hence the axis of the dimer lies along the crystallographic axis. We processed the data on the fly and used scala with the "polish unmerged" output option to find the sites with ShelxD. Five out the 8 expected sites were found and refined, although it was almost impossible to decide the hand, given the low solvent content. The starting resolution was 2.0Å, but the crystal decayed quite soon to 2.5Å.

The second and the third crystals (actually the last two to be collected during the shift) were better diffracting (1.6Å and 1.9Å), but also decayed quite soon to 2.4Å and 3.0Å respectively, despite only 10% of the beam was hitting them. This time the cell was apparently triclinic with cell dimensions (in both cases) of a=40.23Å, b=40.54Å, c=49.49Å, α=92.49°, β=87.79°, γ=109.22°.

Another SeMet protein was tested for MAD, the DNA transcription factor DNR from *P. aeruginosa* expressed in *E. coli*. We tested 4 crystals all with dimensions around 20-30μm. Unfortunately none of them diffracted better than 3.5Å so we did not collect any data, despite a good incorporation of reduced Se was demonstrated by the scan. The same protein, but without the SeMet, had been crystallised in the presence of Cu and Zn, and then back-soaked. The spectrum did not detect any metal incorporation so we did not perform any data collection, since a native at 2.0Å had been already collected in a previous shift.

After that we performed three data collections on the R21L mutant of GST from *S. mansoni* expressed in *E. coli*. The first crystal was grown in the presence of GSH at pH 7.0, the second in the absence of GSH at pH4.0 and the third in the presence of the inhibitor hexyl-GSH at pH7.0. In the table below there is a summary. The three structures have been solved by MR using the wild type protein as template and are currently under final refinement and validation.

	R21L – GSH (pH7.0)	R21L (pH4.0)	R21L – GTX (pH7.0)
wavelength	0.918393	0.918393	0.918404
Space group	$C222_1$	P3	P3
Unit cell dimensions	a=b=74.2Å, c=35.1Å	a=b=53.58Å, c=143.34, γ=120°	a=b=53.29Å, c=142.95, γ=120°
Resolution	30.0-1.9Å	30.0-1.7Å	30.0-1.43Å
Completeness	99.1%	99.6%	98.0%
Multiplicity	4.0	2.6	3.8
Rsym	5.2%	5.1%	7.1%

Then we tested, without success, 13 tiny crystals of XendoU, an endo-ribonuclease from *X. laevis* expressed in *E. coli*, and grown in the presence of UMP and Mn^{2+}, in order to get a data set with resolution better than 2.3Å, before finding one which was diffracting to 1.9Å. According to strategy, 120° would have been enough to have a 98% complete data set. After 60° the diffraction decayed to 2.8Å, so we centred in another zone, given the elongated shape of the crystal, and started again from the 30^{th} frame for another 30 frames. While collecting the third of these passes we experienced the shutting off of the front end and the lost of the first (of course the best) images, hence very unfortunately at the end the crystal was not long enough to have a complete data set.
After this data collection we tested 3 crystals of the F112L mutant of a DPS from *L. monocytogenes* expressed in *E. coli* seeking for a resolution better than 2.5Å, and none was collected, as well as 2 micro-crystals of an Endo-glucanase from *B. subtilis,* expressed in *E. coli*, which proved to be salt crystals. Other two crystals of EryK, a cytochrome P450 from S. coelicolor expressed in E. coli and co-crystallised with its inhibitor klotrimazol, were again tested but none of them diffracted better than 2.9Å so were not collected, since a data collection at 2.3Å was already performed in a previous shift.