Final Report of MX332 at ID14-3

Several data sets have been collected at ID14-3 with a classical non automated way of changing samples despite unfortunately experiencing a three hours block of the collection time due to RF failures of the storage ring.
Overall the beamline is very user-friendly and the system of automatic centring of the crystal is very good, even in terms of zoom-power, brightness and optics. One minor point which we could arise is the impossibility of checking the crystal status during data collection. In fact it happened that one crystal (mounted on a pin that was maybe not exactly of the same size of the others) apparently decayed but in fact moved out of the beam. So we had to restart the data collection, instead of throwing away the crystal.
We started the shift by testing and then collecting the best of the crystals of a mutant of Glutathione S Transferase (GST) with and without substrates. The crystals were too small to be tested with home sources and in fact most of them diffracted poorly even with synchrotron light. The best data collection, which lead to the structure by molecular replacement, was called R21L (from the name of the mutation) and its statistics are shown in table 1:

R21L (resolution 1.9Å)	Data reduction
Space group	$I2_13$
Unit cell dimensions	a=b=c=150.014Å; α=β=γ=90°
Mosaicity	0.456
Completeness (2.11-1.9Å)	100% (100%)
Multiplicity (last shell)	11.85 (12.4)
Rsym (last shell)	0.113 (0.351)
B (Wilson)	30.1
	Refinement (50.0-2.0Å)
R	0.21
Rfree	0.25
r.m.s.d. bond length	0.0087
r.m.s.d. bond angle	1.04

Table1 summary of data from R21L mutant of GST

Since the wild type crystallised in a $P2_1$ space group and $I2_13$ could come from twinning, we checked the cumulative intensity distribution (Fig. 1).

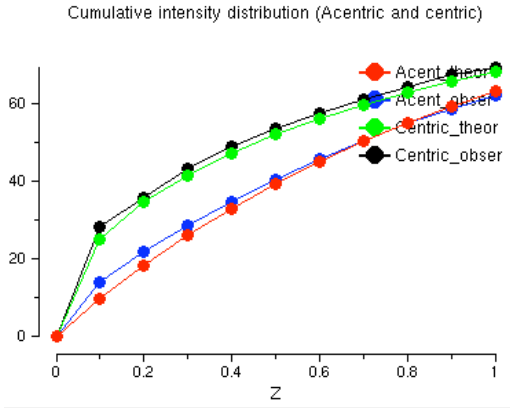

Fig.1 Cumulative intensity distribution for R21L

Indeed the mutation dramatically changed the space group, although from a preliminary inspection of the dimer interface the number of contacts are apparently the same. Some external loops are less structured than the wild type, given the looser packing of the cell.

After the break we collected for the first time a data set of the protein DNR, a transcription regulator sensing NO. This very first crystal gave a good native data set up to 1.9Å and the results are summarised in Table 2. Since this is a new structure of its family, we could not go further in structure solving, without any derivative.

DNR	Data reduction
Space group	C2
Unit cell dimensions	a=55.62Å, b=105.64Å, c=74.82; β=98.19°
Mosaicity	1.2
Completeness (2.11-1.9)	98.2% (89.8%)
Multiplicity (last shell)	5.3 (4.8)
Rsym (last shell)	0.103 (0.213)
B from Wilson	19.2

Table2. Summary of data from the gene regulator DNR

Then we collected one good native data set of the protein EryK, a cytochrome P450 involved in the biosynthetic pathway of polyketide antibiotics. Preliminary data were collected at 2.8Å and revealed the presence of a long axis. In fact the data collected on a tiny crystal of 50x20x20μm at 1.81Å with a $\Delta\phi$=0.1° confirmed what known.
At present the structure is going to be solved by MR using another component of the family (EryF, PDB code 1OXA) sharing 25% sequence identity with EryK. Preliminary maps are interpretable, but the structure needs rebuilding in many areas. A summary of the data collection is given in Table 3 and a picture of the area surrounding the heme in Fig. 2.

EryK	Data Reduction
Space group	$P2_12_12_1$
Unit cell dimensions	a=37.93, b=57.34, c=179.76
Mosaicity	0.51
Completeness (1.91-1.81Å)	89% (87.7%)
Multiplicity (last shell)	4.5 (2.6)
Rsym (last shell)	0.19 (0.75)
B from Wilson	17.9

Table3. Summary of data from cytochrome P450 EryK

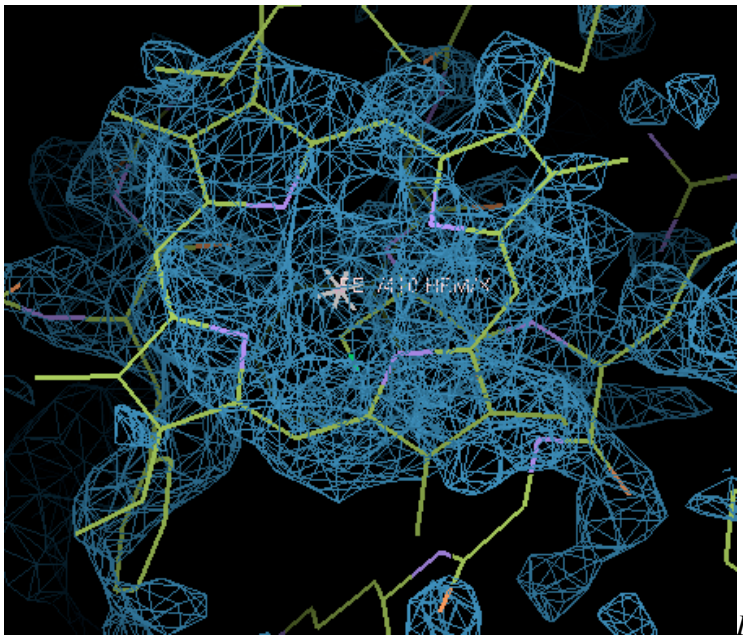

Fig.2. A portion of the first map after MR, obtained with another cytP450 as a search model.

Furthermore, we collected the first native data set of the polygalacturonase (PG) from the phytopathogenic fungus *Colletotrichum Acutatum*. Unfortunately crystals were very small so we could collect a data set only at 2.7Å resolution.
Data statistics were pretty good and a summary of the data collection is reported in the Table 4 below:

PG	Data Reduction
Space group	$P2_12_12_1$
Unit cell dimensions	a=85.85, b=128.55, c=207.47
Completeness	95.6%
Rsym	0.17

Table 4. Summary of data from C. acutatum PG

Towards the end of the shift we tested several crystal of a bacterial GST soaked with different antibiotics, such as rifamycin, tetracycline, and phosphomycin. Unfortunately, even the shortest soaks introduced disorder within the cell (P4) leading to very high anisotropic data or to multiple lattices. We shall try to perform cocrystallisation hoping to be more successful.