Report of Experiment MX608 at ID29 on 11 may 2007

During the shift assigned to our bag we used the sample changer and unfortunately we had a lot of problem, some of which we were able to solve by looking at the trouble shooting web-page, while others required the local contact assistance.
The changer worked well only whit Hampton research *HP vials - loops*.
We used DNA only for testing, we collected manually and processed the frame using moslfm.
The beam intensity was fine during the whole shift except for a couple of hours during which there were problems whit the computers network of the whole synchrotron.
We found extremely helpful and scrupulous the assistance of our local contact Dr David Flot who was precious and very fast in problem solving.

We tested 38 crystals, and performed 12 data collections of crystals belonging to 5 different projects.

1) Lamirinase endo-beta-1,3 glucanase from Pyrococcus furiosus is an enzyme which displays its main hydrolitic activity on the beta 1,3 glucose polymer laminarine. We tried to collect two MAD datasets on this protein but we did not succeed. The first crystal reported serious radiation damage during the second data collection (at the inflection point).
The second ones diffracted at low resolution and was twinned.
In any case, we processed the first dataset and we used it for SAD phasing.
The dataset collected at the peak of the Selenium X-ray fluorescence spectrum is 95 % complete at 2.6 Å resolution with an Rmerge of 9.3 %.

2) Norcoclaurine synthase (Nausica) catalyzes the condensation of dopamine and 4-hydroxyphenylacetaldehyde (4-HPPA) as the first committed step in biosynthesis of benzylisoquinoline alkaloids in plants. Many of these compounds are pharmacologically active, including the analgesic and antitussive drugs morphine and codeine, the antibiotic sanguinarine.
The aim of our project is to solve the structure of Nausica from Thalictrum flavum in order to understand the structural basis of Nausica enzymatic activity.
We already collected a 99 % complete dataset at 2.8 Å resolution on a crystal soaked with dopamine. The measured crystal is primitive trigonal (space group P31) with the following cell dimensions : a=b=86.127 Å, c=117.595 Å.
Since we failed to solve the structure by Molecular Replacement, we are trying to solve the structure by MIR. During this shift we collect three datasets; the first on a crystal soaked with K_2IrCl_6 and the other two on crystals soaked with K_2OsO_4.
The best data collection was measured on a crystal soaked with K_2OsO_4 is 97.7 % complete at 2.8 Å resolution with an Rmerge of 8.2 %

3) We aimed to solve the crystal structure of the flavodiiron proteins (FDP) from the protozoo *Giardia lamblia* in the reduced form. We tested many crystals reduced anaerobically with different amount of dithionite but unfortunately most of them did not diffract. Finally we tempted to collect a data sets at 3.2 Å resolution. Unfortunately data collection was not complete because of the poor quality of the crystal.

4) In the symbiotic, N_2-fixing bacterium *Bradyrhizobium japonicum* N oxide-mediated induction of the denitrification *nir* and *nor* genes is under the control of the transcriptional regulator NnrR, belonging to the CRP-FNR superfamily of regulators. To date no structural information is available on the regulators involved in N-oxides

dependent activation of the denitrification patways in bacteria.
During this shift we were able to collect a native data set at 2.8 Å resolution and three data set on crystals soaked with Au, Hg and Pt which diffracted at 3.2, 3.5 and 4.2 Å respectively and were isomorphous with the native crystal.

NATIVE - NnrR () – ID29

Space group	P212121
Unit cell dimensions	a=55.42 b=88.44 c=101.47
Resolution (Å)	66.5-2.8
Mosaicity	0.80
Rsym (last shell, 2.8 Å)	0.061 (0.651)
Completeness (last shell) %	98.9 (98.4)

ISOMORPHOUS DERIVATIVES NnrR – ID29

Heavy metal	**Au**
Wavelength (nm)	1.000
Resolution (Å)	101.5 – 3.2
Rsym (last shell, 3,1 Å)	0.110 (0.352)
Completeness (last shell) %	99.8 (100.0)
Heavy metal	**Hg**
Wavelength (nm)	1.000
Resolution	101.5 – 3.5
Rsym (last shell, 3,1 Å)	0.080 (0.353)
Completeness (last shell) %	99.9 (100.0)
Heavy metal	**Pt**
Wavelength (nm)	1.000
Resolution	101.5 – 4.2
Rsym (last shell, 3,1 Å)	0.073 (0.537)
Completeness (last shell) %	99.0 (98.5)

5) Gpx (glutathione peroxidase) from *Schistosoma mansoni.* We tried to solve the sturucture of the enzyme in complex with GSH. We collected two data set at 1.4 Å and the second from 40.0 to 1.04Å (in 2 passes); GSH was not incorporated and active site Cys was oxidised to Sulfenic acid.

space group	P212121
cell dimensions:	a=41.37
	b=60.36
	c=62.62Å
refinement	R=0.135
	RFree=0.162
total protein atoms	1426
waters	135

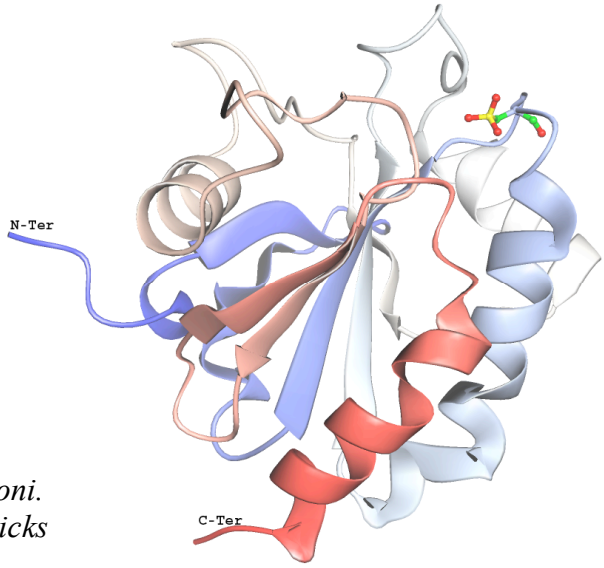

Figure 1. *Gpx from Schistosoma mansoni. Active site Cys is shown as balls and sticks*