Report of Experiment MX608 at ID14-2 on 19 september 2007

During the shift assigned to our bag we used the sample changer.
We lost the first 3 hours of the shift since the sample changer was out of order and had to be reinitialized an cooled again. After this initial problems the changer worked properly and except for 2 or 3 samples that were not unmounted (we used only Hampton research *HP cap and vials*).
We found extremely helpful and scrupulous the assistance of our local contact Dr Thibaut Crepin who was precious and very fast in problem solving.
We used DNA only for testing, but we collected manually and processed the frame using moslfm.
The beam intensity was fine during the whole shift as well as the detector and all the software of the beam line network.

We tested 38 crystals, and performed 7 data collections of crystals belonging to 5 different projects.

1) 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 one dataset on Se-methionine crystals.

Se-MET DERIVATIVE - NauSeMet - ID14-2			
Space group	P3121		
Unit cell dimensions	a=84.754 b=84.754 c=113.456 gamma=120°		
	Overall	*InnerShell*	*OuterShell*
Low resolution limit	44.00	44.00	4.43
High resolution limit	4.20	13.28	4.20
R_{merge}	0.160	0.060	0.422
Total number unique	3701	138	529
Multiplicity	19.9	11.9	20.9
Mean((I)/sd(I))	23.8	43.5	8.2
Completeness	99.9	97.2	100.0

2) Dissimilative Nitrate respiration Regulator (DNR) from *Pseudomonas aeruginosa* is an NO-dependent regulator which activates the transcription of the enzymes involved in the denitrification pathway. In order to gain insights into the molecular and structural basis of this important regulation. We cloned the *dnr* gene in *E. coli*. The recombinant protein is produced as an homodimer. It is constituted by a sensory domain (N-ter) a dimerization alpha-helix and an Helix-Turn-Helix motif (C-ter).
Recently we solved the structure of a C-ter deletion mutant composed by the sensing domain plus the dimerization helix. We are now aiming at the structure of the full-length protein. We obtained the first crystals from a double mutant DNR-H14A-H15A and during this shift we collected a native data set at 5.5Å

NATIVE - DNR-H14A-H15A - ID14-2			
Space group	P23		
Unit cell dimensions	a=101.86		
	Overall	*InnerShell*	*OuterShell*
Low resolution limit	80.00	80.00	5.80
High resolution limit	5.50	17.39	5.51
R_{merge}	0.627	0.915	0.737
Total number unique	1260	51	181
Multiplicity	19.8	13.7	21.6
Mean((I)/sd(I))	6.4	3.1	2.5
Completeness	100.0	99.2	100.0

3) 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.
We have a native data set at 2.8 Å resolution and three data set on crystals soaked with Au, Hg and Pt which at 3.2, 3.5 and 4.2 Å respectively collected at ID29 in may, but we are still trying to obtain a first set of phases. During this shift we collected a second native data set at 2.8 Å and tested 3 Se-Methionine derivatives which will be collected on a tunable beamline

NATIVE - NnrR – ID14-2			
Space group	P212121		
Unit cell dimensions	a=55.31 b=88.12 c=101.01		
	Overall	*InnerShell*	*OuterShell*
Low resolution limit	48.51	48.51	2.95
High resolution limit	2.80	8.85	2.80
R_{merge}	0.146	0.049	0.838
Total number unique	12732	467	1821
Multiplicity	7.1	5.4	7.3
Mean((I)/sd(I))	12.3	28.2	2.3
Completeness	100.0	99.6	100.0

4) About murine Neuroglobin, we crystallized the protein in complex with CN in order the obtain information on the structure of the globin in a CO bond like state. During this shift we collected 2 data sets on Ngb-CN but only one could be integrated and scaled, the second was not a single crystal and had suffered heavy radiation damage.

Ngb-CN – ID14-2		
Space group	C2	
Unit cell dimensions	a=178.61 b=103.38 c=111.92 beta=104.52°	
	Overall	*OuterShell*
Low resolution limit	29.6	7.3
High resolution limit	7.1	7.1
R_{merge}	0.52	0.86
Total number unique	2498	
Multiplicity	2.8	3.0
Mean((I)/sd(I))	2.7	1.2
Completeness	83.0	87.5

5) Hb-aabb: 20 crystals have been screened for diffraction. The protein is a chimeric construct based on Hba, where the two α chains and the two β chains belong to one polypeptide each, with linkers of opportune length. All the crystals were grown in the CO-bound form, but their intrinsic non-homogeneity resulted in poor diffraction resolution.
Only one crystal was collected because it diffracted at 2.9Å.
The data was 100% complete from 30.0 to 2.9Å with two tetramers in the a.u.; the space group was C2 with cell dimensions a=236.2Å, b=53.9Å, c=136.3Å, beta=123.7°.
The structure has been solved by MR using the program phaser, which placed 4 individual alpha chains and 4 individual beta chains.
The linker on the alpha chains is visible in both tetramers, but the linker between the two beta is disordered; the structure is currently under refinement and improvement of crystal is a work in progress.