Report of Experiment MX497 at ID14.1 on monday 19 feb. 2007

During the shift assigned to our bag we used the sample changer and we had no problem. The changer worked perfectly in automatic mounting and dismounting the crystals. We found the system extremely useful and user-friendly. We used DNA only for testing, we collected manually and processed the frame using moslfm.
The beam intensity was fine during the whole shift.
We found helpful and scrupulous the assistance of our local contact Dr Joanna Timmins.

We tested 28 crystals, and performed 7 data collections of crystals belonging to 5 different projects.

1) 14-3-3 proteins are ubiquitous class of regulatory proteins found in all eukaryotic cells and were the first class of molecules to be recognized as discrete phosphoserine/threonine binding modules. 14-3-3 bind a large number of different substrates to regulate a wide array of cellular signaling events including cell cycle progression and DNA damage responses, programmed cell death, cytoskeletal dynamics.
The aim of our project is to solve the structure of 14-3-3 of Giardia intestinalis in order to understand the structural basis of phosphorylation-dependent binding of 14-3-3 to peptide and protein ligands.
We already collected a 100 % complete dataset at 3.2 Å resolution. During this shift we collected an additional dataset at 3.0 Å, but unfortunately the crystal decayed and data are complete only at 3.3 Å resolution.
The measured crystal is primitive trigonal (space group P31) with the following cell dimensions : a=b=101.10 , c=140.86.
We used this dataset and that previously collected at ESRF to solve the structure by Molecular Replacement.

2) Norcoclaurine synthase (Nausica) catalyzes the condensation of dopamine and 4-hydroxyphenylacetaldehyde (4-HPAA) as the first committed step in benzylisoquinoline alkaloid biosynthesis in plants. Benzylisoquinoline alkaloids are a large and diverse group of secondary metabolites found mainly in five related plant families, including the Papaveraceae and Ranunculaceae. Many benzylisoquinoline alkaloids are pharmacologically active including the analgesic and antitussive drugs morphine and codeine, the antibiotic sanguinarine, and the muscle relaxants papaverine and tubocurarine.
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, c=117.595.
Since we failed to solve the structure by Molecular Replacement, we soaked the native crystals with $Hg(OAc)_2$. During this shift we collected a complete dataset on a Hg soaked crystal at 4.0 Å resolution. The crystal is isomorph with the native ones.
We are still trying to solve the structure with SIR and SIRAS methods.

3) 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 recently cloned the *dnr* gene in *E. coli*. The

recombinant protein is produced as an omodimer. It is constituted by a sensory domain (N-ter) a dimerization alpha-helix and an Helix-Turn-Helix motif (C-ter).
We where not able to obtain crystals of recombinant DNR, thus we expressed a C-ter deletion mutant composed by the sensing domain plus the dimerization helix. We obtained crystals from this truncated DNR and collected a few native data set, phases where obtained by SAD on a Se-met derivative. We are refining the model at 2.1 Å. During this shift we tried to improve the resolution to help the building process but we could only collect a 2.3 Å resolution data set. Space group C2, a=56.71 b=105.96 c=75.34 γ=97.54 - mosaicity 0.4, completeness 99%.

4) Recently, a new thioredoxin reductase was described in animal cells and designated as Thioredoxin/glutathione reductase (TGR). TGR can reduce several components of the thioredoxin and glutathione systems. No structures of TGR are available, but only of its separated domains. This protein was found also in the human parasite Schistosoma mansoni and it was predicted to be a good candidate as drug target; we obtained crystals of the TGR from S. mansoni. During this shift we collected a first data set at 4 Å: C2 space group (unit cell dimensions: a=142.3 b=102.5 c=59.0 γ =112.6). These data were useful to make a successful molecular replacement. We are now trying to improve crystal quality.

5) About murine Neuroglobin, we have cocrystallized this protein in with 10% v/v 1,5-diaminopentane and 10% v/v NAD respectively, in order to explore the affinity of neuroglobin main cavity for non gaseous molecules and to improve eventually crystals quality. We have tested 3 crystals obtained with NAD, subsequently we have selected two of these and collected two relative datasets. Data reduction shows that crystals belong to H32 space group, cell dimentions a=b=88.07 and c=112.7, diffracting at 1.7Å and 1.6Å, both complete at 99%, with redundancy of 10-11% respectively. After first refinement the R and Rfree values are quite good but maps inspection don't shows any ligand molecule interacting with the protein.
We have collected one dataset for crystals obtained with 1,5-diaminopentane; data reduction indicates an H32 space group, cell dimentions a=b=88.5 and c=112.04, higher resolution limit of 1.6 Å; the collection results complete at 99.5% and redundant at 10.7%. Actually these data are under refinement.