Experiment title:

Experiment number: MX-934

Beamline: ID23-eH2

Date of experiment: 28/01/2010 from: 9h30 to:8h00

Date of report: 24/01/2011

Shifts: 3

Local contact(s): Daniele DE SANCTIS

Received at ESRF: Sonia Fieulaine* (CNRS; PhD), Karim Hamiche* (CNRS; Master 2 student), Benoît Gigant* (CNRS; PhD), Marcel Knossow * (CNRS; PhD).

Report:

1) Project 1: saPDF2 (1 shift)
A total of 7 complete data sets were collected, 2 for free enzyme and 5 for soaked ligands. The new model for free enzyme, from crystals grown in new crystallization conditions, was compared to the first model we could solve in a previous experiment. In addition, we could localize in active site 2 new ligands among 4 ligands tested. Structures are refined and the enzymatic characterization of these new drugs has to be finished.

2) Project 2: The structural cycle of tubulin (1.5 shift)
Tubulin is the microtubule (MT) building block. It should be in GTP state to be competent for assembly. MT assembly is accompanied by GTP hydrolysis. During the past decade, we have determined structures of tubulin at 3.5 Å resolution. Whereas these structures have provided insight into the tubulin mechanism, we need more precise structural data to elucidate the structural cycle of tubulin linked to its nucleotide and assembly/disassembly cycles.
In this session, we have started the study of a new crystal form of tubulin in complex with a stathmin-like domain protein. These crystals can be obtained either with GTP or GDP. Whereas the crystals available for this session were rather small, the small size of the beam of the ID23-2 station coupled with the opportunity to translate gradually the crystal during data acquisition permit us to collect complete dataset on several crystals. The best crystals diffracted to 2.5 Å, the statistics being (overall / 2.56-2.5 Å shell): I/sig(I)= 10.9/2.09, completeness 99.4/96.4%, Rfact 8.3/63%, redundancy 4.3/4.17.
The crystals belong to a P222 orthorhombic space group, the cell dimensions being ca. 65 x 128 x 252 $Å^3$. A solution was found by molecular replacement in the $P2_12_12_1$ space group, with one complex in the asymmetric unit. A publication based on the structural results obtained during this and the following sessions is in preparation.