



	Experiment title: BAG - LEBS - 2011-2	Experiment number: MX-1292
Beamline: ID14eh1	Date of experiment: 12/11/2011 from: 9h30 to: 8h00	Date of report: 13/02/2012
Shifts: 3	Local contact(s): P. Carpentier	<i>Received at ESRF:</i>

Names and affiliations of applicants (* indicates experimentalists):

M. Ranaivoson*

M. Knossow*

B. Gigant

Report:

1) Project 1: Tubulin in complex with an engineered stathmin-like domain

Stathmin-like domain proteins from vertebrates bind two tubulin molecules in a T₂SLD complex. We have designed a stathmin-like domain that binds efficiently only one tubulin. Crystals of this assembly further complexed by an anti-tubulin DARPin have been obtained and about 10 crystals have been tested during this session. The diffraction is highly variable. Most crystals diffract to low resolution (about 7 Å). We collected data from two crystals. One of them diffracted to around 4 Å and was not pursued further. The second crystal diffracted to 3.2 Å resolution. Data have been processed in the P1 space group with the following statistics:

Cell dimensions a=58.72, b=59.13, c=155.93, α =84.73, β =86.62, γ =71.70

I/sig = 9.8 (2.0), R_{sym} 9.1% (50%), completeness 96.8% (95.3%), multiplicity 2.5 (2.5)

There are two complexes per asymmetric unit. The structure was solved by molecular replacement and is being refined.

1) Project 2: Tubulin modified with a stathmin-derived peptide

Tubulin readily oligomerizes to protofilament-like assemblies, which makes its structural study difficult by crystallography. We have covalently linked a stathmin-derived peptide at what is termed the longitudinal interface, which prevents this autoassembly. We co-crystallized the modified heterodimer with an anti-tubulin DARPin. 10 crystals of this complex have been tested during the session MX-1292. Most of them diffracted at low resolution (around 7 Å) but some diffracted to better resolution. The best dataset reached (3.7 Å resolution) displayed the following statistics:

Space group P2₁2₁2₁ (a=58.9 Å, b=67.9 Å, c=308.3 Å).

I/sig = 13.9 (1.6), R_{merge} 6.8 % (73.6 %), completeness 98.8 % (96.4 %), multiplicity 4.7 (4.6).

There is one complex per asymmetric unit. The structure was solved by molecular replacement and the preliminary electron density maps show the positioning of the bound peptide.