Experiment title:

Experiment number: MX-1140

Beamline: ID29

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Shifts: 3

Local contact(s): Daniele DE SANCTIS

Received at ESRF: Agata Nawrotek (CNRS, P6 University; PhD student), Benoît Gigant* (CNRS; PhD), Samira Zouhir (CNRS, University Paris-sud 11; PhD student), Sylvie Nessler* (University Paris-sud 11, Pr).

Report:

1) Project 1: The structural cycle of tubulin (A. Nawrotek, B. Gigant)

During this year, we have exploited a new crystal form of tubulin that diffracts to up to 2.1 Å. Crystals of tubulin in different nucleotide states have been collected, which permit us to complete the structural cycle of tubulin related to its nucleotide cycle and to its assembly/disassembly cycle in MT (paper in preparation).

By varying the crystallisation conditions, crystals can be obtained in 1 hour. During this session, we tested crystals obtained in such conditions. Three dataset were collected, two from crystals grown with tubulin in complex with a stable analogue of GTP, one from a crystal further incubated with a 30 residue peptide of a tubulin interacting protein. Statistics are summarized in the following table:

	Resolution (Å)	Completeness (%)	I/sig(I) (last shell)	Rmeas (%)
Crystal 1	2.8	99.7	16.9 (3)	9.6
Crystal 2	2.9	99.7	21.8 (3)	7.3
Crystal 3	3.3	99.9	19.7 (3.1)	8.4

It appears that these crystals are isomorphous to the older ones and the structures are similar. Moreover there is no signal in the electron density maps for the soaked peptide.

2) Project 2: Crystals of a new tubulin assembly (A. Nawrotek, B. Gigant)

We have engineered stathmin domain-based proteins able to bind more than two tubulins. We have obtained crystals of these new tubulin complexes and tested ~12 of them during this session. Most of them diffract to low resolution (less than 4.5 Å), and the diffraction is anisotropic. One crystal was significantly of better quality, and a dataset to 4.2 Å resolution has been collected. As one cell parameter is about 640 Å, we collected very thin images (0.1° per image) as it is possible with the Pilatus detector now installed on ID29. The structure has been solved by molecular replacement. This fixes the stoichiometry of the complex, which has remained ambiguous from biochemical experiments.

3) Project 3: Quorum sensing effector NprR (S. Zouhir, S. Nessler)

NprR in complex with its cognate NprX peptide

We recently solved at SOLEIL a medium resolution (3.6Å) structure of a complex between a truncated form of NprR and a 7aa long form of its cognate NprX peptide, using selenomethionine labeling and SAD phasing. We now try to solve a high resolution structure of this truncated form of NprR in complex with different forms of the NprX peptide.

We tested around 16 different crystals.

One of them diffracted up to 3.4Å resolution and allowed us to collect 1 complete data set:

The crystal diffracted in space group P1 (a=120.35Å, b=133.39Å, c=136.73Å, α=108.519°, β=104.396°, γ=103.91) with good statistics (Rmerg= 0.13, data 90% complete, I/sig= 12.92 (1.7)). The first structure was used as initial model for molecular replacement, allowing us to fix the orientation of the peptide, which has

remained ambiguous in the medium resolution density map.

Apo-form of truncated NprR

We also tested around 10 crystals of the apo-form of NprR truncated but they all diffracted at very low resolution (around 10Å) and no data sets were collected with these crystals.

4) Project: T3SS effector SlrP (S. Zouhir, S. Nessler)

A truncated form of SlrP in complex with human thioredoxine

Three data sets had already been collected on ID23-1 (07/23/2010) at 3.5Å resolution.
They were of poor quality and did not allow us to solve the structure by molecular replacement using the available structures of two homologous T3SS effectors.
We used the Pilatus detector recently installed on ID29 in order to obtain new data of better quality and try again.

	Resolution (Å)	Completeness (%)	I/sig(I) (last shell)	Rmeas (%)
Crystal 1	3.35	99.1	18.26 (3.36)	5.6
Crystal 2	3.6	98.5	13.10 (2.9)	8.3
Crystal 3	3.6	100	14.6 (2)	6.9
Crystal 4	3.6	100	15 (2.1)	7.3
Crystal 5	5.45	100	15.8 (2.5)	11.6

Among 20 crystals tested, 5 were used for data collection:
Crystals diffracted in space group $P2_12_12_1$ (a=106.29Å, b=135.21Å, c=155.61Å).

These new data sets finally allowed us to obtain a solution with molecular replacement.
We are now trying to find out if thioredoxine is present in this new structure.