ESRF	Experiment title: **Data collection of native FtsY in complex with GDP, GTP analogs and of the mutants D449N and T331A**	Experiment number: Ls-688
Beamline: BM-14	**Date of experiment:** from: 19. Sept. to: 21. Sept. 97	**Date of report:** 20.Okt.97
Shifts: 6	**Local contact(s):** Andy Thompson	***Received at ESRF:***

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Report:

The structure of the FtsY, the Signal Recognition Particle (SRP) docking protein has been solved recently in our lab (1). The protein appears soluble in the cytoplasm, but also attached **to** the membrane. The mechanism of interaction with the SRP and with the membrane is still not clear. Since GTP binding and hydrolysis to FtsY play an essential role in the regulation of the SRP cycle, we are very interested in the structure of the protein-nucleotide complex.

Two data sets of FtsY-mutants have been collected. One for the D449N mutation, which changes the specificity from GTP to XTP (xantinetriphosphate), and a second one for T33 1 A, the Thr 33 1 has been proposed to play an important role in the coordination of the Mg and the γP during nucleotide hydrolysis. In the apo structure T331 was found to be positioned far away from the location of the corresponding T35 in Ras (for details see: 1, 2).

l.- The first data set was collected for **FtsY** co-crystallized with a GDP. The data could not be collected at home due to the small size of the crystals (soaking of the native crystals with different nucleotide analogs didn't work). The space group and cell constants didn't vary with respect to the apo structure (P21 a=325 b=79.5 c=59.3 β=94.6) the data set was 96 % complete with an Rsym of 5.8 % and the resolution limit was 2.6 Å.

2.- The second data set was collected on the XTP mutant co-crystallized with XMPPNP. Again the data were not collected at **home** (for the same reason as above). The space group and cell constants didn't vary with respect to the native apo structure (P21 a=32.S b=79.5 c=59.3 β=94.6) the data set was 98 % complete with an Rsym of 8% and the resolution limit 2.4 A. The structure **has been** refined **with an Rfactor** of 21% and an Rfree of 27 %. The position of the side chain of N449 is similar to the D449 in the native structure but no nucleotide could be detected in the pocket.

3.- A data set for the mutant T331A was collected, The crystals were also quite small for a home source. The space group was different to the wild type P21212 with cell axis a=79.2 b=107.7 and c=32.3 Å. the data set was 99 % complete with an Rsym of 7.8% and the resolution limit 2.7 Å. Structure determination b y molecular replacement and refinement is underway.

Ref:

1. Montoya, G., Svensson, C., Luirink, J. & Sinning, I. (1997) Nature, 385, 365-368.
2. **Moser, C.,** Mol, O., Goody, R.S. & Sinning, I. (1997) *Proc. Natl. Acad. Sci.* USA 94, 11339-l 1344.