



ESRF
experimental
report

Experiment title:

Data collection on crystals of EF-Tu.GDPNP:Phe-tRNA

**Experiment
number:**

LS-17 (#76)

**Beamline(s)
used:**

Beamline 4- ID 2

**Date and time of
experiment:**

from: 19/06/94

to: 20/06/94

**Local
contact(s):**

B. Rasmussen

Name and affiliation of applicants (please mark
experimentalists with an asterisk):

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Date of report

29/08/94

received
(completed
ESRF): by

0 4 MAR 1996

Experiment report *(If this work has been published, please give reference and abstract):*

Two datasets were collected at app. 125 K to at least 4.0 Å resolution. Maximum resolution observed was 3.5 Å. Exposures at 277 K showed the same maximum resolution. The size of crystals used for datacollection was 0.1 x 0.2 x 0.4 mm.

The data were recorded images covering 1 degree of crystal rotation with an exposure time of 60 seconds. The crystal to image-plate distance of 400 mm and the size of the beam was limited to 0.2 by 0.2 mm by the adjustable apertures of the MAR.

The spacegroup were determined to be C2 with $a=220.0$ Å, $b = 127.1$ Å, $c = 158.6$ Å and $\beta = 129.2^\circ$. The effective mosaic spread was 0.8° . Processing of the data have so far been unsuccessfull as it has been impossible to scale the frames.

In a parallel experiment at Daresbury lab. station 9.6 data were collected to 2.8 Å with 90 sees/degree exposure time, crystal size 0.15 x 0.4 x 0.6 mm, slits 0.3 x 0.3 mm and the effective mosaic spread was 0.35° . A rough estimate is that the beam at ESRF BL4/ID2 is 15 times more intense than station 9.6 at Daresbury.

The poor diffraction of the crystals was first attributed to the cryosystem which could not go lower than 125 K as measured on the crystal position. As the exposure at 277 K was made we had to realize that the crystals had been damaged prior to flash freezing. We found, however, that at 125 K it was impossible to flash freeze a thin film of cryobuffer without having ice formation. This had previously been done successfully at our home lab. at 100 K. Had the crystals had not been damaged the cryosystem might have been the limiting factor.

The crystals are grown at 4° C and are temperature sensitive. During transport the crystals are kept on ice. At ESRF we chose to keep the crystals on ice near the beamline, rather than using a distant coldroom at EMBL, and only remove them from ice-boxes for very short periods when transferring crystals to cryobuffer. This procedure may have been inadequate for keeping a reasonable constant temperature and thereby causing damage to the crystals.

The crystals used at ESRF where grown two weeks prior to the crystals used at Daresbury and we suspect that crystal age also affects the quality.