ESRF	**Experiment title:** Crystallography of Ribosomes	**Experiment number:** LS-235 Commissioning
Beam]ine: ID2	**Date of Experiment:** from: 23. Nov. 95 to: 24. NOV. 95	**Date of Report:** 6.10.96
Shifts: 3	**Local contact(s):** J- Shaw / B. Rasmussen	*Received at ESRF:*

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Report:

Three shifts were assigned to us in order to establish the feasibility of data collection from ribosomal crystals at the high brilliance station BL4(ID2) and to demonstrate its superiority over the second generation stations which are being used by us. Our specific aims were:

- to check the geometrical parameters at the crystal environment, including the suitability of the available space for the entire cryo equipment we brought with us, focusing on the stringent requirements of the crystal transfer machine (the liquid propane flipper), the visibility of crystals mounted on the double glass spatula on the video screen and the possibility to irradiate crystals at various locations;

- to assess the time needed for obtaining useful data in order to plan the future measurements. This task includes studies of crystal decay by radiation damage in view of the need to change crystals while collecting a data set, which, in our case is a time-consuming process because of the need for screening for suitable crystals in view of the low level of homogeneity and isomorphism of the ribosomal crystals. These searches could be reduced or even eliminated if we could irradiate different parts of the crystals until they decay;

to assess the gain in useful resolution limits under the experimental conditions at BL4 (such as the brightness of the beam, the geometrical parameters of the station and the type of detector) and compare them with those obtained at BW6 and BW7 at DESY and at F1/CHESS:

Six native and derivatized crystals of the large ribosomal subunits from from Haloarcula marismortui were exposed. All experiments were performed at cryo temperature (about 90 K), using our own N_2 cryo-temperature equipment which was left at ESRF for further experiments as well as for other users. Our preference for using the equipment designed by us is based on previous experiments. performed at CHESS and DESY, which clearly showed that in terms of resolution limits and crystal life-time our design is more suitable than the commercial ones (e.g. Oxford Cryosystem, and to lesser extent, the MSC system).

To minimize the time wasted for crystal screening, we brought with us several pre-frozen crystals, which were preliminary partially characterized at station BW6/DESY. We also intended to assess the feasibility of transferring irradiated frozen crystals to ESRF without loss of resolution.

For performing our experiments some adjustments and fine tuning of the station were required. These operations lasted for about half a shift in the beginning of our measuring time. In addition, the beam stop had to be improved and this task was performed during our last shift. All together more than one shift was dedicated for these manipulations and improvements. Hence, our real experimental time can be considered as less than two shifts. As a result of these efforts, station BL4 (lD2) is currently suitable for data collection from ribosomal crystals and its superiority over the second-generation synchrotrons was clearly demonstrated even during this short "commissioning time" experiment which was performed under many restrictions. Some detail is given below.

We found that despite the small cross-section of the bright BL4 beam, since a large MAR detector was installed, we could not obtain data to higher than about 4.5 Å resolution without translating the detector sidewise or vertically. These limits were dictated by the crystal-to-film distance required for reasonable spatial separation of our crystals due to the large unit cell, 580 Å, and the fairly high mosaicity, up to 1.3 degrees. Since realistically including these translations of the detector in our beam-time was not possible due to the time-shortage and some engineering difficulties, we performed our studies at this resolution limit and focused on derivatives* which are supposed to phase at comparable resolution.

The most striking observation is the gain in exposure time. Thus, only 3-10 minutes were needed for collecting one frame (with a rotation of 1 degree) at the experimental maximum resolution (4.5 Å). compared with 40-60 minutes which were required in BW6/DESY for collecting the data to 8-9 Å. which seemed to be the maximum resolution for this particular crystal at that station.

It was shown that the rate and severity of crystal decay depends on its initial resolution. Thus, at 4.5 Å a serious crystal decay occurred with in the first 8 exposures (each of 3-6 minutes), whereas less observable decay was evident even after a total of 40 minutes of irradiation of a crystal which diffracted originally to 7 A only. Soaking ribosomal crystals in "radiation suppressors" did not removed or ease the decay, suggesting that the damage the crystal suffers at cryo temperature is not directly related to the propagation of free radical. However, the alternative way to overcome part of the radiation damage was more successful. Thus, despite the small size of our crystals and the use of spatula for mounting them, with some sophistication it was possible to translate from the beam-path the part of the crystal which was damaged and measure the parts which were not irradiated earlier. The "freshly exposed" regions were found not to be harmed by the previous experiments. Thus, more data could be collected from a single crystal.

From one crystal, soaked in $Cs_7(P_2W_{17}O_{61}Co(NC_5H_5))nH_2O$, we collected about one quarter of a data-set (23 degrees, in two locations). We found that the evaluated data are satisfactory despite the beam sensitivity and the experimental constrains. Another derivated crystals (with $(K_5O_2)_3(PW_{12}O_{40})$), was used earlier for data collection in Hamburg an in experiment which was pre-maturely terminated when the crystal started to show slight decay. Further fast decay was observed in Grenoble within the first few exposures, which included centering and alignment. Therefore no data could be collected from it.

*$K_{14}NaP_5W_{30}O_{110}$, $(NH_4)_6(P_2W_{18}O_{62})14H_2O$, $(K_5O_2)_3(PW_{12}O_{40})$, $Cs_7(P_2W_{17}O_{61}Co(NC_5H_5))nH_2O$