EUROPEAN SYNCHROTRON RADIATION FACILITY

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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** **Nanocrystallography of biological cells**	**Experiment number**: MX-1036
Beamline: ID13-1	**Date of experiment**: from: 22 April 2010 to: 2 December 2010	**Date of report**: 7 February 2011
Shifts: 12	**Local contact(s)**: Dr. M. Burghammer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. D.S. Eisenberg; Dr. R. Riek;

***Dr. C. Reikel ; *Dr. B. Brumshtein; *Dr. J.P. Colletier ; *A.Soragni**

Report:

In our proposal we suggested investigating x-ray diffraction from fibrillar and nano-crystalline deposits that naturally occure in granulocytes (purified core particles) of human *eosinophiles* and *Drosophila Melanogaster* larva crystal cells. In view of a small size of fibrillar and crystalline deposits, approximately ~1μm^3 or less, to date ID-13 is the only beamline capable of producing a suitable x-ray beam of 1μm^2, or sub-micron size, for our purpuses. We find that these type of experiments are quite new and therefore, they initially focused on assessing our ability to obtain an x-ray diffraction images of deposits that show a high degree of symmetry and naturally appear in living cells, and also to evaluate effects of various sample preparation techniques on their ability to diffract.

Experimental setup: Due to the nano-meter size of our samples, we found that in order to record the weakest possible reflections, it is crucial to obtain the highest possible sensitivity and to reduce effects of background scattering. Therefore, the experimental setup invovled a secondary aperture; it was prepared of a lead sheet and mounted between the collimator and the beamstop. The apperture was skillfuly installed by our local contact, Dr. Manfred Burghammer to whom we are greateful for help and suport with our experiments. All samples were previously cryo-cooled and applied in a stream of 100K nitrogen gas.

Sample preparation: All sample were mounted onto various types of macromolecular crystallography cryo-mounts and were frozen in either liquid nitrogen or propane; several cryo protecting solutions were tested. Our findings suggest that the optimal freezing procedurs include: 1. Samples of e*osinophil* granules provide

best results if concentrated and frozen in a nitrogen streem, immedietely prior to their application, and MiTeGen kapton loops are the most suitable for their mounting (Fig. 1). 2. *D. Melanogaster* cells containing crystalline deposits were genetically marked with a fluorescent protein in order to ease on identification and centering, it was performed under a laser illumination (Fig. 1). MiTeGen kapton grids with 10μm aperture seemed to produce best results, and freezing in liquid propane reduced formation of ice.

X-ray data collection: In case of *eosiniphil* granules, static x-ray diffraction images were collected in a mesh scanning mode, with steps of 2μm. Such a technique was applied since it is not possible to observe fibrillar deposits inside granules under a light microscope. Therefore, we had obtain images separated by periodic distances, thus attempting to identify points of interest. Images which showed increased low resolution scattering were further analyzed for diffracted reflections (Fig. 2). Similar mesh scans were performed over the fluorescent *D.Melanogaster* cells. As in *eosinophil* granules, images that exhibited a increased low resolution scattering were further analyzed.

Preliminary results: X-ray diffraction image of eosinophil granules showed three major arc-shapped reflections at resolution of 4.8Å, 48Å and 59Å. *D. Melanogater* cells showed reflections at 4Å, 26Å and 39Å. We are currently analyzing results and would like to perform more experiments, collect more data on our existing samples and try specimens with other naturally appearing crystals and fibrils inside cells.

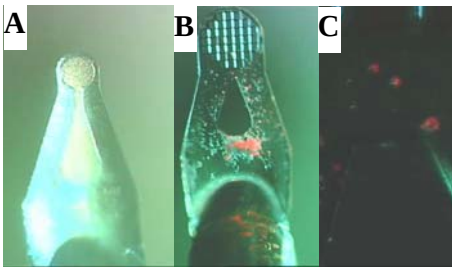

Fig. 1. **A.** concentrated *eosinophil* granules. **B.** *D. Melanogater* lavra cells marked by fluorescent protein marker. **C.** fluorescent cells with low background light.

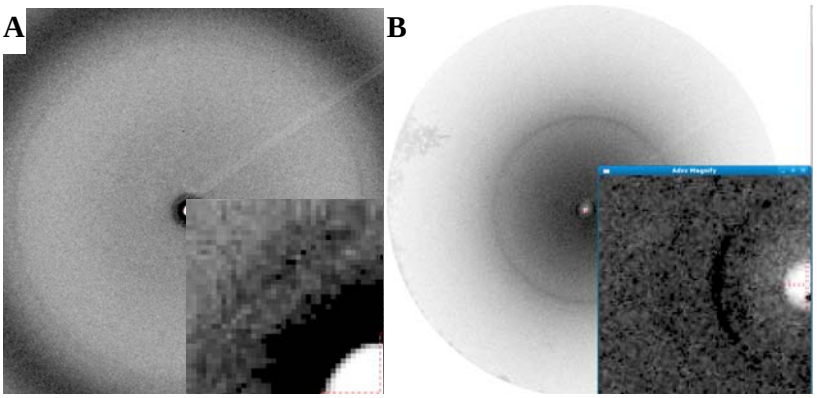

Fig. 2. Some of the x-ray diffraction images obtained at MX-1036. **A.** X-ray diffraction of crystal containing *eosinophil* granules. Relfections appear at 4.8Å and 59Å. **B.** X-ray diffraction reflections of *D.Melanogaster* crystal cells. Insert shows magnification of low-resolution reflections. Reflections were recorder at 4Å, 26Å and 39Å.