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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-1296
Beamline: ID13-3	**Date of experiment**: From: 09/11/2011 to: 12/11/2011	**Date of report**: 28/02/2012
Shifts: 12	**Local contact(s)**: Dr. M. Burghammer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Alice Soragni* – ETH Zurich, Institute of Physical Chemistry, Wolfgang-Pauli Strasse 10, 8093 Zurich, Switzerland (present address: UCLA-DOE Genomic Center, 611 Charles E. Young Dr. East, Los Angeles, CA-90095 - USA)

Christian Riekel* and **Manfred Burghammer*** - European Synchrotron Radiation Facility, 6, rue Jules Horowitz, F-38043 Grenoble Cedex 09

Jacques-Philippe Colletier* - Institut de Biologie Structurale, J.P. Ebel, 41, rue Jules Horowitz , F-38027 Grenoble Cedex 01

David Eisenberg, **Boris Brumshtein*** and **Michael Sawaya*** - UCLA-DOE Genomic Center, 611 Charles E. Young Dr. East, Los Angeles, CA-90095 - USA

Report:

Experimental setup. The experiment was performed at the ID13 nanofocus hutch (EH-3) with the monochromatic beam focused to a spot of ~200(h)*150(v) nm. Ag-behenate was used as a calibrant. Images were acquired with a Medipix pixel detector that combines a fast readout with a high sensitivity. Samples were in this case deposited on silicon nitride membranes and dried, since the measurements were performed at room temperature. We raster scan the silicon nitride membranes to localize the single crystals.

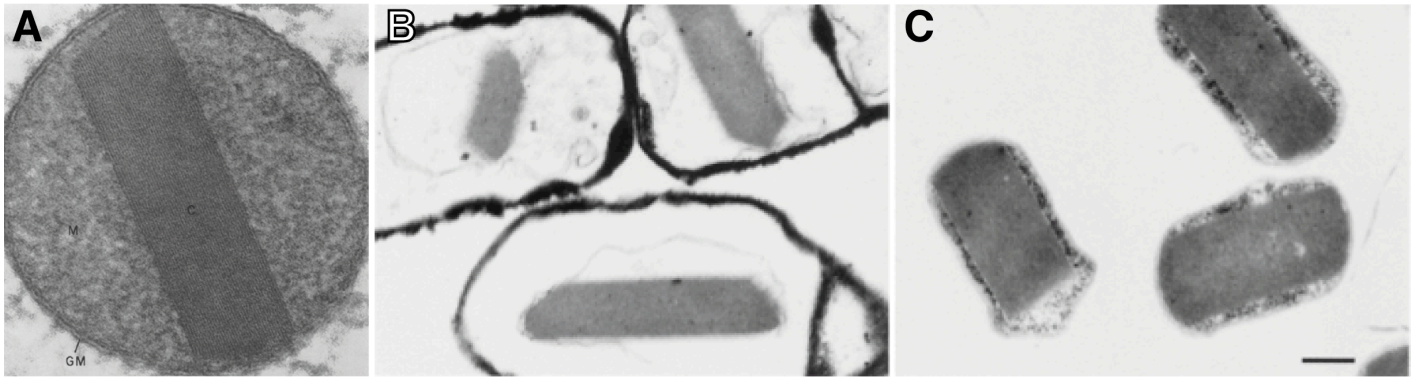

Fig. 1. A. EM picture of a thin section of a granule from a rat eosinophil infiltrating the intestinal mucosa magnified 375.000x. Reproduced from Miller F. et al., J. Cell Biol. 1966. **B.** EM pictures of *B. thuringiensis* containing crystalline inclusions. Images reproduced from Appl Environ Microbiol. 1998 October; 64(10): 3932–3938.

Samples: Eosinophils. The sample preparation is identical to that of the experimental report MX-1192. Briefly, eosinophils were isolated from healthy donors/hypereosinophilic patients and granules containing crystals were extracted from the cells with established methods (Fig. 1A), frozen in a sucrose/ethylene glycol solution and defrosted just before the measurements directly at the ESRF in Grenoble. Additionally,

crystalline cores were extracted from granules upon treatment with a mild detergent (Triton 0.1%), spun through a sucrose cushion and frozen in a sucrose/ethylene glycol solution. Both granules and cores were thawed at the ESRF, extensively washed in water to remove any salt, resuspended in water and deposited on silicon nitride membranes (Silson). To concentrate the samples further, multiple layers were deposited and dried on each membrane. ***Bacillus thuringiensis.*** *Bacillus thuringiensis* cells containing crystalline deposits of the Cry1A and Cry3A proteins were used. Wild type strains of *B.thuringiensis* produce crystalline inclusions of proteins that appear to be natural toxin to pests (Fig. 1B and 1C). They appear as a well ordered crystals with approximate dimensions of ~1μm across (Fig. 1). Current variants of *B.thuringiensis* were genetically modified and over-produced Cry1A and Cry3A proteins, resulting in increased crystal sizes. For the experiment, *B.thuringiensis* cells were mounted onto MiTeGen micro mounts.

X-ray data collection: X-ray diffraction images were collected in a mesh scanning mode, with steps of 500 nm to few microns. Over 50,000 diffraction images were recorded. Such a technique was applied since it is not possible to visualize the granules or crystals using the in-line light microscope due to their small size. Therefore, we had to use the approach to raster scan large areas of the membranes to identify points of interest. Images of static samples were collected and processed. Reflections were detected with Fit2D software.

Preliminary results and conlusions: Eosinophils. Remarkable progress was made in characterizing diffraction from eosinophil nanocrystals. In this experimental session we examined two kind of samples, intact granules and isolated crystals. The latter, had a tendency to show powder diffraction, most likely due to the aggregation of several crystals in the drying process. The resolution limit of the powder diffraction patterns extended to approximately 15 Å. The intact granules measured in the last two shifts showed a better dispersion, and single crystal diffraction was attained. We speculate that the granules are protecting the crystals from dehydrating during the hours required to complete the experiment at ambient temperature and help to avoid co-aggreation of different crystals. This information is valuable to us because future experiments can be focused on the granules, rather than cores. Two of the best single-crystal diffraction patterns from eosinophil granules are shown in Fig. 2.

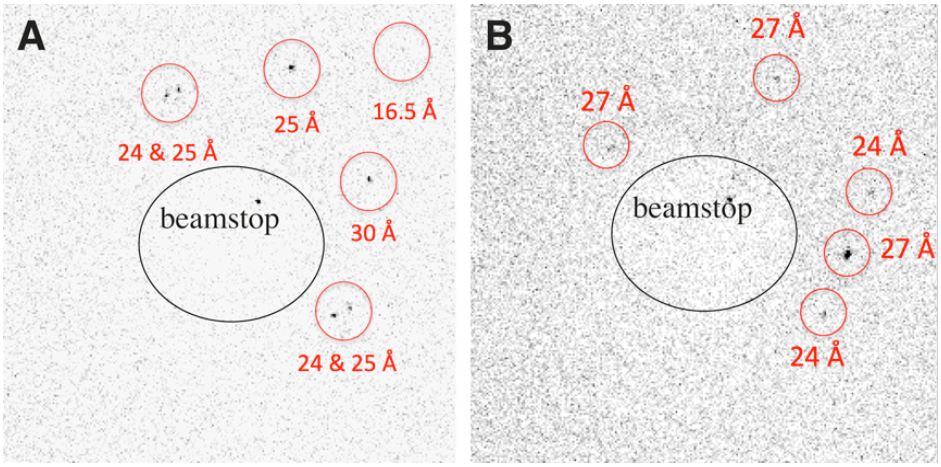

Fig. 2. A. and **B.** Selected diffraction patterns recorded during a mesh scan on a granule sample dryed on a silicon nitride membrane. Several reflections are visible. The resolution of the diffraction is indicated in red.

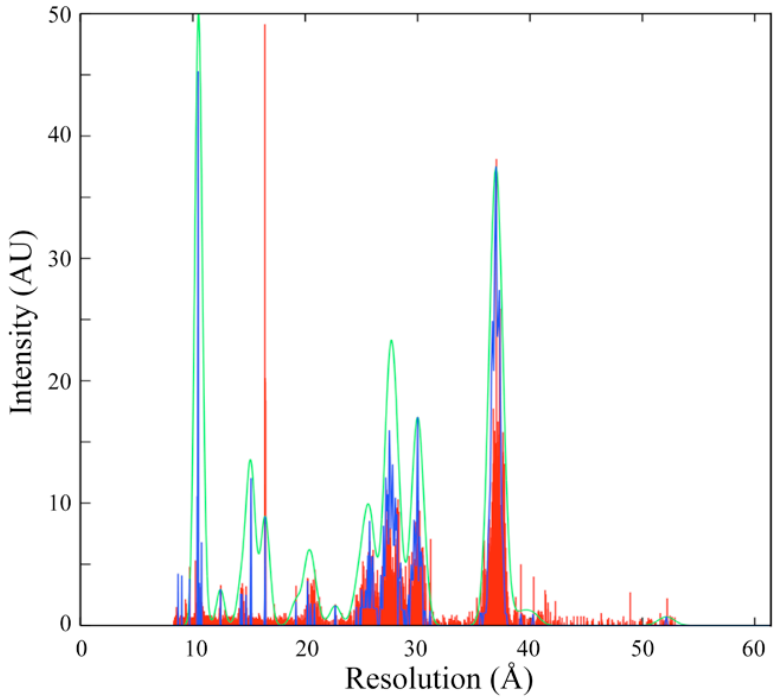

Fig. 3. Analysis of the observed reflections from all isolated cores (blue) or the gaussian fit for the same data (green) and intact granules (red). I/σ is plotted as a function of resolution.

Data analysis reveals that crystal parameters are well correlated between the purified cores and the granules (Fig. 3). There is agreement in the Bragg spacings and intensities as shown in Fig. 3. The red lines indicate the intensities and resolution of the reflections recorded from granules. The blue lines indicate analogous quantities for powder diffraction from the purified cores. The correlation in unit cell parameters is not surprising since the crystals have the same origin. The correlation indicates the quality of the diffraction experiment and the robust nature of the nanocrystals.

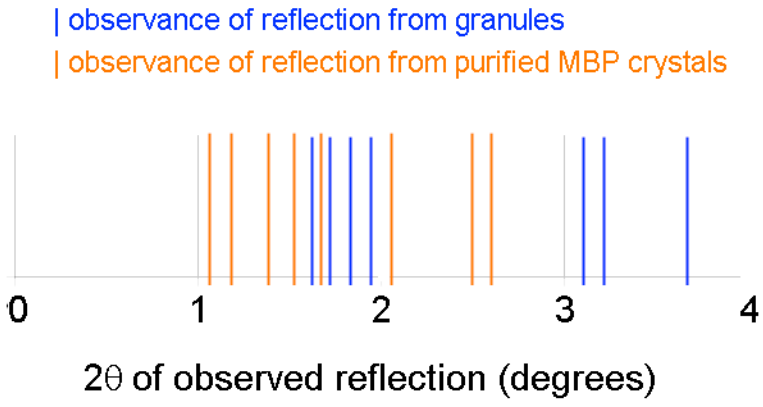

Fig. 4. Comparison of the observed reflections obtained from the granules and from purified MBP artificially crystallized *in vitro*.

The diffraction patterns provide us with further evidence that the naturally occuring crystals of MBP made by the eosinophil have different parameters than those obtained from the recombinantly express MBP protein. In Fig. 4, we illustrate the poor correlation between the two-theta values of the reflections recorded from the granules (blue) and those collected from the recombinant protein (orange). The results suggest that the crystal

structure reported for purified MBP does not directly inform us of the lattice contacts present in the naturally ocurring crystals.

Bacillus thuringiensis. Preliminary data analysis for the *B.thuringiensis* diffraction patterns allowed us to estimate the unit cell parameters. For indexing and Bravais lattice determination only reflections with $I/\sigma > 5$ were considered. Low resolution reflections were not used for the purpose of analysis, since shapes of reflections showed a large variability. Additionally, small positional deviations resulted in large differences in measured reflection angles; therefore only reflections with resolution higher than 30Å were used (Table 1). Possible unit cells were fitted to reflect the data with allowed dimensions smaller than 500Å, and the obtained values were: a=374 Å, b=75 Å, c=351 Å, β=109 (Table 2). Achieving X-ray diffraction data from crystalline deposits within *B.thuringiensis* cells validates that the chosen methodology can be applied for studying material in cells however the diffracting quality of the eosinophil crystal exceeded the *B.thuringiensis* samples that will therefore not be studied in further detail.

Table 1. Measured x-ray Bragg reflections

d(Å)	I*	2Θ(OBS)
29.6	12	0.157
21.0	15	0.222
18.3	20	0.254
16.6	56	0.280
7.5	12	0.621
5.8	14	0.804
* all reflections showed $\langle I/\sigma \rangle > 5$ Lower resolution reflections were not used for Bravais lattice calculations due to deviations in spot shapes and imprecision in 2Θ measurements.		

Table 2. Results of indexing of x-ray reflections.

Bravais lattice: P Monoclinic **Unit cell: a=374 Å, b=75 Å, c=351 Å, β=109** ***Rp=0.17**					
H	**K**	**L**	**2Θ(OBS)**	**2Θ (CALC)**	**DIFF**
10	0	-10	0.157	0.158	0.001
10	0	10	0.222	0.222	0.000
20	0	-10	0.254	0.255	0.001
0	0	20	0.280	0.280	0.000
0	10	0	0.620	0.621	0.001
40	10	-20	0.802	0.803	0.001
* Rietveld profile R factor (H.M. Rietveld, J. Appl. Cryst. 2 (1969) 65-71)					