## EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON



# **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:  Cellular receptor recognition by measles virus	Experiment number: MX-85			
Beamline: ID14-4	<b>Date of experiment</b> : from: 5/7/03 to: 7/7/03	<b>Date of report</b> : 9/07/2003			
Shifts:	Local contact(s): Dr. Didier NURIZZO	Received at ESRF:			
Names and affiliations of applicants (* indicates experimentalists):  *César Santiago, Karolinska Institute.  *José M Casasnovas, Centro Nacional de Biotecnología-CSIC.					

### Report:

Data collection was planned at the tunable beam line ID14-4 with native and derivative (Se and Br) crystals of the measles virus hemagglutin protein complex to its CD46 receptor. Crystals belong to the orthorombic P2221 space group. Intensive work was carried at the beam line during the 48 hr of allocated time, 40 to 50 protein crystals were tested during the experiment, and 11 complete datasets were collected with 6 different crystals:

- 1.- Initially we started collection of X-ray diffraction data with Se-Met derivative crystals. Presence of Se-Met was confirmed by the fluorescent scan performed with all three crystals used in data collection. Crystal damage because of radition was quite aparent after about 180 seconds (30°) of exposure. Crystals were then moved along its long axis for successive exposure, allowing completion of data sets. Four complete data sets (3 at the peak and 1 at the inflection point) were collected with three different crystals. Data sets were processed with mosfilm and scala and gave good statistics for resolutions ranging from 100 to around 4 Å (I/ $\sigma$ > 2 at high resolution). So that a significant improvement from data (6 Å) collected in another synchrotron facility was achieved. Significant anomalous signal from 20 to 4.5 Å was observed from two data sets collected at the peak . Search of the Se-Met sites is now underway.
- 2.- A second set of experiments were done using native crystals, some of which were treated with glutaraldehide and/or grown in the presence of several cations for

improvement of crystal diffraction anysotropy. Quite unexpectingly most of our native crystals did not difract at resolutions beyond 5 Å. From about 25 tested crystal a complete data set was collected, bearly reaching 3 Å resolution (I/ $\sigma$  about 1.5 at the highest resolution beam). Slight resolution improvement from our past data (3.2 Å) was achieved, although a higher resolution was initially aimed.

3.- A third set of experiments was carried with crystals grown in the presence of NaBr. Crystals prepared with two hemagglutinin protein variants were tested. They were washed in cryoprotectant solution lacking NaBr prior to flash freezing. Presence of Br in the crystals was monitored by a fluorescent scan, which provided absortion edges. The crystals appeared more resistant to radiation damage than Se-Met crystals, so that complete data sets were collected at three different wavelengths (peak, inflection point and high-energy remote) with two different crystals. One of those crystals were prepared with a different protein variant, giving similar space group and unit cell. Processed data had good statistics for resolutions ranging from 100 to 3.9 Å. Search of Br sites is underway.