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.

	Experiment title: Structural proteomics on Mycobacterium tuberculosis	**Experiment number**: 14-U-855
Beamline: BM14	**Date of experiment**: from:11/11/2006 to:12/11/2006	**Date of report**:
Shifts: 3	**Local contact(s)**: Hassan Belrhali	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Inaki de Diego * (EMBL Hamburg outstation, Notkestrasse 85, 22603 Hamburg, Germany)

Jochen Kuper * (EMBL Hamburg outstation, Notkestrasse 85, 22603 Hamburg, Germany)

Matthias Wilmanns (EMBL Hamburg outstation, Notkestrasse 85, 22603 Hamburg, Germany)

Report:

Crystals of a DAPK/ Ca Calmodulin (CaM) complex were taken along as a test case in order to assess their diffraction properties at an ESRF beamline. The intensity of the DORIS ring in Hamburg was not sufficient to provide useful data although it could be verified that crystals were protein crystals indeed. The presence of both proteins in the crystals has been verified beforehand by SDS-PAGE analysis revealing clearly single bands for the DAPK and CaM. Making use of the sample changer robot about 20 crystals were checked for their diffraction properties on BM14. Eventually, a 2.5Å data set could be collected and processed. The space group is the primitive orthorombic $P2_12_12_1$ with a= 45.5, b=101.9, c=105.3. The space group is similar to the previously solved kinase domain of DAPK but with a significant larger unit cell already indicating the possible presence of CaM. The processing statistics are given in table 1. The structure was solved on site by MR using the entry 1JKL that represents the complete kinase domain of DAPK (residues 1-277). Searching for CaM with various different CaM structures with bound petides did not yield a solution for CaM. After manual rebuilding of the kinase part it was obvious that more of DAPK was visible in the electron density maps. The additional residues 277-318 could be placed manually leading to significant improvement of the phase information. Additional electron density became visible indicating the presence of CaM. After manually building 5 of CaM helices (2 N-terminal and 3 C-terminal) the two Calmodulin lobes could be placed manually. However, the density for many parts of CaM remain poorly and show very high B-Factors indicating partial disorder or still poor very heavily biased phases from

MR. The model with the complex could be refined to r- values of 24/31 (r and r-free) using refmac5. Although the presence of CaM could be clearly verified better data is needed in order to exploit all the implications this important complex structure can show us. The crystals tested on BM14 clearly showed that using an even stronlger beamline the diffraction can be enhanced. Radiation damage has not been observed so far.

Table 1. data collection statistics:

	Data collection
Wavelength (Å)	1.02
Max. Resolution (Å)	2.5
Space group	$P2_12_12_1$
Unit cell (Å)	46.5 101.9 105.3 90 90 90
Completeness†	99.9(100)
Mean Redundancy	3.8(3.8)
R_{sym}†‡	6.6 (43.4)
I/sigI†§	9.6 (3.85
Number of reflections	19927

For integration and scaling mosflm and scala have been used.