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:	Experiment number:
ESRF	Crystal structure of DAPK in complex with Ca/Calmodulin	MX631
Beamline:	**Date of experiment**: from: 17/12/2007 to: 18/12/2007	**Date of report**: 28/08/2007
Shifts:	**Local contact(s)**:Edward Mitchell	*Received at ESRF:*

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Report:

Crystals of a DAPK/CaM complex that has been previously characterized in experiments at BM14 were taken to ESRF beamline ID29. Since the structure was essentially solved with the BM14 dataset the main objective of the experiment was to enhance the resolution and reliability of the current working model. This task was followed via two approaches. 1) Getting a native data set at the highest resolution possible. 2) Obtaining a data set at a wavelength that will enable us to see the anomalous signal of Ca in CaM in order to get an unbiased verification of the proposed model.

Summary to approach 1):

Indeed we were able to obtain better resolution data without radiation damage that resulted in a complete data set at 2.1Å resolution collected at wavelength of 1.23Å. Statistics of this data set are given below. The refinement was finished using the previously acquired model from the BM14 data set. The final statistics of the refinement process are also given below. Most importantly the data resulted in a highly improved density for the N-terminal part of CaM that is partly disordered and was only poorly visible previously.

Summary to approach 2):

A complete highly redundant data set has been collected at a wavelength of 1.77Å up to a resolution of 2.7Å. The previously obtained model for the high resolution data set was used for refinement against this set yielding the statistics given below. Using the anomalous data of this set we could clearly identify all 4 Ca sites in Calmodulin. Thus we could verify our atomic model of the CaM position with independent data largely excluding model bias for the overall position of CaM.
In summary the provided beamtime has been a great success for us in the task of elucidating the molecular mechanisms of kinase regulation by the general regulator CaM.

Table 1 data collection statistics

Data collection statistics		
Data set DAPK/CaM	Hi-res	anomalous
Wavelength (Å)	1.27	1.77
Max. Resolution (Å)	2.1	2.7
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit cell a,b,c (Å)	46.4 101.7 104.8	46.5 101.9 104.8
α,β,γ (°)	90	90
Completeness†	99.9(100)	99.2(98.5)
Mean Redundancy	3.4 (3.5)	11.2(11.5)
$R_{sym\dagger}$†‡	9.4 (68.4)	11.9 (57.5)
I/sigI†§	9.2 (1.8)	40.3 (3.8)
Number of reflections	29723	14205

Table 2 Refinement statistics

Refinement statistics		
Data set DAPM/Cam	Hi-res	anomalous
Resolution limits (Å)	52-2.1	50-2.7
Number of reflections	28185	13436
R_{cryst} / R_{free} (%)¶	19.5/ 26.9	22.3/ 30.6
Deviations from ideal values in		
Bond distances (Å)	0.016	0.009
Angles (°)	1.534	1.124
Torsion angles (°)	6.415	5.245
Chiral-center restraints ($Å^3$)	0.088	0.079
Plane restraints (Å)	0.005	0.003