

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 studies of carbohydrate-modifying enzymes (additional project related to application MX-170)	Experiment number:
Beamline: ID29	Date of experiment: From: 16/4/2003 to: 18/4/2003	Date of report: 10/12/2003
Shifts: 6 (shared with MX94)	Local contact(s): Andrew McCarthy	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): Sine Larsen Leila Lo Leggio* Heidi A. Ernst* Eva Johansson*		

Report:

A report has already been submitted for the projects contained in the original application. However during the allocated shifts for MX93 some data was collected on one of the projects described in proposal MX170. At the time of application for MX170 we had not yet collected data for the present proposal MX93, hence we could not know that the time available at ID29 could cover both projects.

Heavy atom derivatives data was collected for crystals of the NAC binding domain of *Arabidopsis* ANAC. ANAC belongs to a large family of plant specific transcription factors. The data collected at ID29 has allowed structure determination and resulted in an article describing crystallization (Olsen *et al* in press) and a manuscript describing the structure (Ernst *et al.*, submitted). Abstracts for the two manuscripts are enclosed below.

Olsen, A.N., Ernst H.A., Lo Leggio, L., Johansson, E, Larsen, S and Skriver K. "Preliminary crystallographic analysis of the NAC domain of ANAC, a member of the plant-specific NAC transcription factor family" *Acta Crystallogr. D.* in press

Abstract

The NAC domain (residues 1-168) of ANAC, encoded by the abscisic acid responsive NAC gene from *Arabidopsis thaliana*, was recombinantly produced in *E. coli* and crystallized in hanging drops. Three morphologically different crystal forms were obtained within a relatively narrow range of conditions: 10-15% PEG4000, 0.1 M imidazole/malic acid buffer pH 7.0 in the reservoir, 3.2-7.7 mg ml⁻¹ in the protein stocks, and 1:1 ratio of reservoir to protein solution in the hanging drop. One of the crystal forms, designated crystal form III, was found to be suitable for further X-ray analysis. Form III crystals belong to space group $P2_12_12_1$ with unit cell parameters $a = 62.0 \text{ \AA}$, $b = 75.2 \text{ \AA}$ and $c = 80.8 \text{ \AA}$ at 100 K. The cell volume is consistent with two molecules in the asymmetric unit, and a peak in the native Patterson map suggests the presence of a non-crystallographic two-fold axis parallel to a crystallographic axis. Size exclusion chromatography of the NAC domain showed that the dimeric state is also the preferred one in solution, and probably represents the biologically active form. Data sets from four potential heavy atom derivatives of the form III crystals were collected. The derivatized crystals are reasonably isomorphous with the non-derivatized crystals and the four data sets are being evaluated for use in structure determination by Multiple Isomorphous Replacement.

Ernst, H.A., Olsen, A.N., Skriver, K, Larsen, S and Lo Leggio, L. "Structure of the conserved domain of ANAC, a member of the NAC family of transcription factors" submitted to EMBO Reports

Abstract

The structure of the DNA-binding NAC domain of *Arabidopsis* ANAC (abscisic acid responsive NAC) has been determined by X-ray crystallography to 1.9 Å resolution (PDB codes 1UT4 and 1UT7). This is the first structure determined for a member of the NAC family of plant specific transcriptional regulators. NAC proteins are characterized by their conserved N-terminal NAC domains that can bind both DNA and other proteins. NAC proteins are involved in developmental processes including formation of the shoot apical meristem, floral organs and lateral shoots, as well as in plant hormonal control and defence. The NAC domain does not possess a classical helix-turn-helix motif, instead it reveals a new transcription factor fold consisting of a twisted β-sheet surrounded by few helical elements. The functional dimer formed by the NAC domain was identified in the structure, which will serve as a structural template for understanding NAC protein function at the molecular level.

