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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:** Crystallographic structure determination of the bacteriophage T4 short fibre	**Experiment number**: LS-1922
Beamline: ID14-4	**Date of experiment**: from: 5-10-2001 7am to: 6-10-2001 7am	**Date of report**: 27-4-2002
Shifts: 3	**Local contact(s)**: R. Ravelli	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***Ellen Thomassen**

***Mark van Raaij**

Report:

Introduction

Bacteriophage T4 is an elementary lifeform that displays a striking action: after recognising its bacterial host, it latches on and punctures the cell wall to inject its DNA, like a self-powered hypodermic syringe. T4 has an elongated head with icosahedral ends, containing its double-stranded DNA. For injection, it uses a contractile tail.

Adsorption of bacteriophage T4 to *Escherichia coli* is mediated by long and short tail fibres. Long fibres are responsible for initial, reversible, attachment, after which the short fibres extend and bind irreversibly to lipo-polysaccharide core and serve as inextensible stays during DNA-injection. Short tail fibres contain an N-terminal virus binding domain, a shaft and a C-terminal receptor-binding domain.

Previous work

Bacteriophage T4 short fibre protein (gene product 12) was co-expressed in *E. coli* with its chaperone gene product 57 (1). Folding experiments showed an unfolding transition at 56 degrees Celsius (2). Proteolysis of partially unfolded gp12 in the presence of EDTA identified a stable fragment of 33 kD (3) of which the structure was subsequently solved. This 33 kD fragment misses the N-terminal virus-binding domain and the C-terminal receptor-binding domain. Later proteolysis experiments with gp12 partially unfolded in the

presence of zinc ions identified a larger, 45 kD, fragment with an intact receptor-binding domain, which could also be crystallised.

Aims

Our aims were:

1. to collect a high resolution native dataset of the 33 kD fragment
2. to collect MAD datasets for better phasing
3. to test the recently obtained crystals of the 45 kD fragment

Results at this visit

Aim 1: High resolution data collection of the 33 kD fragment. A complete and high-quality dataset was collected to 1.7 Angstrom resolution. Refinement of the previously solved bacteriophage T4 short tail fibre 33 kD fragment structure against this data is in progress.

Aim 2: Several MAD datasets were collected around the Hg-edge. One was also collected around the Cu-edge as one of the crystals appeared to contain copper as judged from fluorescence measurements. However, after data processing we did not obtain evidence for an ordered copper atom. The Hg-MAD datasets are currently used to obtain better phases. However, preliminary electron density calculations show no better density for the disordered N-terminal region and the focus has since shifted on the structure determination of the larger fragment (see aim 3).

Aim 3: Tests of recently obtained crystals of the 45 kD fragment. Five crystals were tested, of which four showed fibre diffraction, indicating the short fibre protein is not three-dimensionally ordered in these crystals. However, one crystal showed, apart from extensive diffuse scatter, a clear lattice. Diffraction spots were observed to around 1.5 Angstrom and a complete dataset was collected to 2.3 Angstrom resolution. However, we could not solve its structure by the molecular replacement technique and will need to collect derivative data. We hope the same mercury derivative used for the 33 kD fragment will also successful for structure solution of the 45 kD fragment.

Note: Structure solution of the 45 kD fragment has recently indeed been achieved. See the report of experiment 14-U-37.

References:

1. Burda MR, Miller S. Folding of coliphage T4 short tail fiber in vitro. Analysing the role of a bacteriophage-encoded chaperone. Eur J Biochem 1999 Oct;265(2):771-8
2. Burda MR, Hindennach I, Miller S. Stability of bacteriophage T4 short tail fiber. Biol Chem 2000 Mar;381(3):255-8
3. van Raaij MJ, Schoehn G, Jaquinod M, Ashman K, Burda MR, Miller S. Identification and crystallisation of a heat- and protease-stable fragment of the bacteriophage T4 short tail fibre. Biol Chem 2001 Jul;382(7):1049-55
4. van Raaij MJ, Schoehn G, Burda MR, Miller S. Crystal structure of a heat and protease-stable part of the bacteriophage T4 short tail fibre. J Mol Biol 2001 Dec 14;314(5):1137-46