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: Bacteriophage MS2 mutant coat protein complex with bacteriopgage Q RNA	**Experiment number**: MX 133
Beamline: ID14-EH1	**Date of experiment**: from: 26 February 2004 to: 27 February 2004	**Date of report**: 30-Aug-04
Shifts:1	Local contact(s): Sofia Macedo	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):
T. Alwyn Jones, Uppsala University
*Kaspars Tars, Uppsala University, kaspars@xray.bmc.uu.se
Lars Liljas, Uppsala University, lars@xray.bmc.uu.se

Report:

Small RNA phages, belonging to the family *Leviviridae* have been used extensively as models for studies of various problems in molecular biology, including protein-RNA interactions, repression of translation, virus assembly and virus evolution.
The capsids of small RNA phages have T=3 symmetry and can be regarded as built up from 90 dimers. Dimers of the viral coat protein bind to a specific site on the viral RNA. This binding is thought to be the initiation of assembly, and the specificity in the binding leads to encapsidation of the correct RNA molecule. The binding is also used in regulation of the expression of the replicase gene. The coat protein acts as a repressor of the translation of this gene. The coat protein dimer binds to a stem-loop structure including the initiation codon of the gene. This binding is very specific and coat protein from one phages species normally do not recognize RNA from other similar phages.
The three dimensional structure of bacteriophage MS2 has been determined earlier. The genomic RNA is invisible in the crystal structure of native MS2 since upon crystallization particles will have different orientations. Also, in native phage the specific coat protein - RNA interaction occurs only in one place per casid. However, the MS2 crystals of recombinant, “empty” capsids can be soaked in mother liquor, containing RNA stem-loop fragments. The RNA then diffuse inside the capsids and binds to all (or most) of 90 equivalent binding sites.
The three dimensional structure of bacteriophage MS2 coat protein in complex with its native RNA binding fragment has been determined earlier using the method described above.
Our collaborators have earlier isolated MS2 coat protein mutants, capable to bind RNA of anoter small RNA phage Q with 1,000-fold stronger affinity than wild-type MS2 coat protein.

We collected a single 2.8Å dataset of MS2 mutant coat protein crystals, soaked with RNA fragment from another small RNA phage Q. The solved structure confirmed the presence of Q RNA and provided some explanations of why mutant coat protein has stronger affinity to Q RNA. As already expected from base-pairing predictions, there are three nucleotides in the loop, compared to four nucleotides in MS2 RNA. It also appears that RNA stem is able to form two distinct conformations with and without a buldge. One of the mutated residues N87A leaves more space for the purine base in Q RNA, explaining increased affinity. Other mutation E89K eliminates negative charge repulsions between aspartate and RNA phosphate. At present, structure is refined and writing of the paper is in progress.