EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** Binding of resolvase enzymes to DNA junctions	**Experiment number**: MX1033
Beamline: ID 14-3	**Date of experiment**: from: 28-11-2009 to: 30-11-09	**Date of report**: 24 March 2010
Shifts: 6	**Local contact(s)**: Cyril Dian, Petra Pernot	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr John Rafferty (University of Sheffield)*

Report:

The experiments aimed to give preliminary data on the solution structures of complexes of Holliday junction resolvases bound to their DNA Holliday junction substrates. They were focussed upon 3 enzymes – *B.subtilis* RecU, *E.coli* RusA and *L.lactis* phage67 RuvC. The intention was to collect data on the free enzymes and examine their solution profiles as well as that of their DNA complexes because this was the first time that the SAXS technique had been applied to these systems. Crystal structures were already available for the free enzymes and thus a comparison could be made with the SAXS model envelopes. In addition the crystallographic work on the RecU enzyme had provided a structure in which a small N-terminal domain was not visible and it was possible that the SAXS data might give some insight on the structure of the full protein. Earlier data from gel-based electrophoretic mobility assays (EMSAs) had suggested that the structure adopted by the Holliday junction, a common substrate for all the enzymes, was different in each case upon binding protein. Thus it was hoped that a comparative study would be possible.

All samples had been shipped frozen in advance to the synchrotron including the components for buffer correction measurements. This was the first time that the experimental team had carried out any SAXS measurements and much of the first shift was taken up with initial sample preparation and familiarisation with the automated

sample changer equipment and the controlling software. We were grateful for the assistance and guidance of the local contact at this time but this was a weekend beamtime allocation and he was unable to stay beyond the first half of the shift, although he did provide telephone contact details.

Data were collected on each of the free enzymes in turn using the equipment in translational 'flow' mode to minimze the effects of radiation damage which could be monitored by comparison of the slope of plots from repeat runs. The available standard SAXS software was used for data processing including the GNOM program at the beamline. Guinier plots were computed for each free sample (Fig.1) and the expected molecular weights for the free enzymes from the SAXS data agreed well with the predicted values of those of the enzyme dimers based upon their sequences.

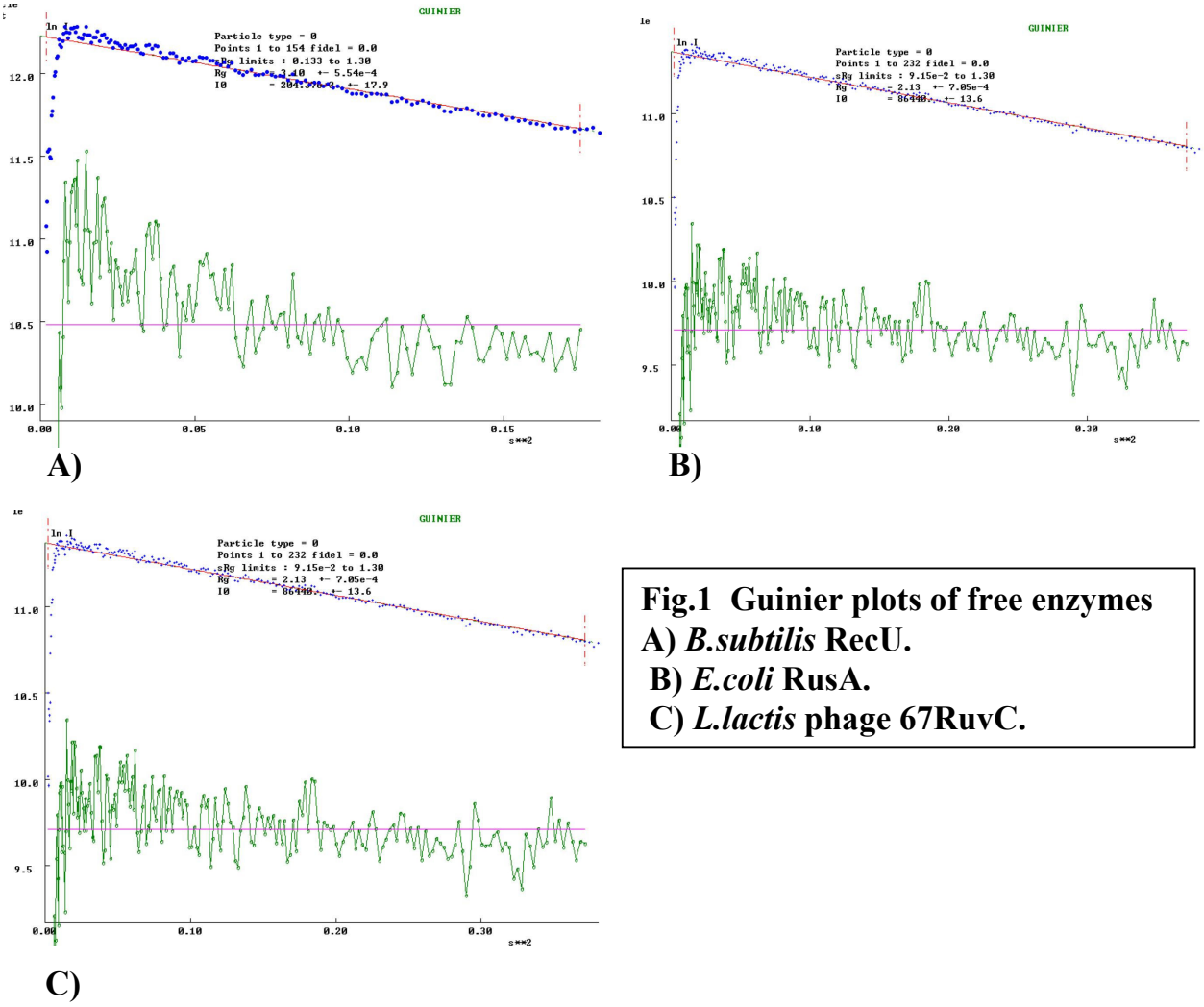

Fig.1 Guinier plots of free enzymes
A) *B.subtilis* RecU.
B) *E.coli* RusA.
C) *L.lactis* phage 67RuvC.

It was possible subsequently at home using the Atsas software suite following re-processing with GNOM to analyse the data with the PRIMUS program and to compute envelope models with the GASBOR and DAMMIN programs for the three enzymes. A range of models were calculated and their fit to the crystal structures noted. An example for RecU is shown (Fig.2) and a reasonable agreement between the crystal and SAXS models could be seen for the other free enzymes.

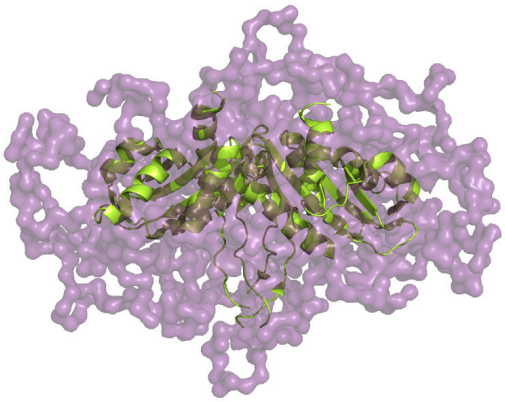

Fig.2 RecU structure. Example fitting of X-ray crystal structure to a SAXS envelope.

However, the data from the complexes with the Holliday junction DNA was of much poorer quality and has been largely ascribed to poor sample concentration coupled perhaps to incorrect buffer background corrections. An example of the Guinier plots for a Holliday junction complex is given in Fig.3 and one can see that in particular even in this best case, the low angle data is very poor. Efforts to compute SAXS model envelopes produced stuctures that made very little structural sene and emphasized the need to collect better quality data.

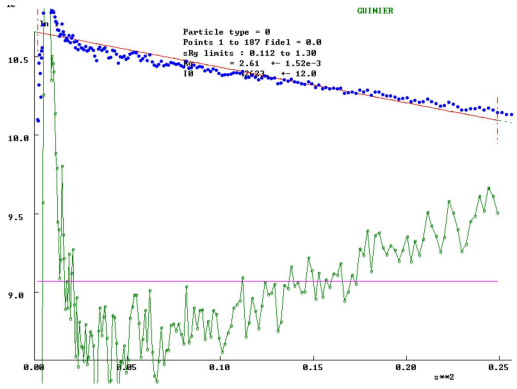

Fig.3 Holliday junction complex Guinier plot. The best plot from the data collected on complexes of a resolvase with a synthetic Holliday junction construct. The low angle data is of particular concern due to its low quality, which is probably because of sample concentration problems.

Thus we proved that the enzyme samples are of sufficent quality and stability to be shipped readily and good data leaing to acceptable models can be collected from them. We are now seeking further time at the ESRF and elsewhere to pursue the DNA complex studies using the knowledge from our first attempts.