EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

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:** Determination of the Quaternary Structure of key proteins involved in *Plasmodium falciparum* pathogenesis	**Experiment number**: MX1080
Beamline: ID14-3	**Date of experiment**: from: 7/12/2009 to: 8/12/2009 (3 shifts) (MØ) from : 23/4/2010 to 24/4/2010 (2 shifts) (LZ) from: 1/7/2010 to 1/7/2010 (1 shift) (AR)	**Date of report**:
Shifts: 6	**Local contact(s)**: Mats Økvist (MØ), Louisa Zerrad (LZ), Adam Round (AR)	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Stig Christoffersen, University of Copenhagen *

Annette Langkilde, University of Copenhagen*

Sine Larsen, University of Copenhagen*

Report:

The aim of this experiment was to use Small Angle X-ray Scattering to determine the overall structure of two proteins belonging to the family of *Plasmodium falciparum* erythrocyte membrane proteins 1 (PfEMP1). The PFEMP1 proteins are expressed on the surface of the infected erythrocytes (IE) and serve as adhesion ligands that interact with a range of host cell receptors.

Experiments performed:

The experiments were conducted at three different visits to the ESRF.

The first visit took place 7 December 2009, and showed that the experiences gained during at MX1073 in terms of sample preparation and operation of the beamline had been most valuable. Measurements were carried out on a carefully prepared and purfied sample of VAR2CSA at five different concentrations. Measurements were also performed for the antibodies that had been shown to specifically bind to the DBL3 and DBL5 domains of VAR2CSA, and for the 1:1 complexes of the antibodies and VAR2CSA. The SAXS measurements of the antibodies were very satisfactory showing that these were pure, and had a molecular structure of the expected size and shape. However the measurements on the complexes revealed that these had aggregated in our solutions. Similar difficulties (aggregation) with the samples encountered on measurements of complexes of VAR2CSA and its carbohydrate targets CSA and decorin. Unfortunately the beamline lost alignment after injection, which had an effect on the last measurements.

The beamtime made available for the second visit was significantly reduced, the ash cloud in Iceland caused a delay of the airplane. Due to the difficulties encountered with measurements on complexes with antibodies different approaches were used to assign the specific location of domains in the overall structural envelop of VAR2CSA. As we expected that the binding of fab fragments would be more specific we performed measurements on complexes of VAR2CSA and the fab fragments for DBL3 and DBL5. Though the measurements were improvements compared to the complexes with the full antibodies, the solutions of the complexes also showed aggregation. The other approach namely to perform measurements on truncated versions of VAR2CSA turned out to be more successful. We performed measurements at three different concentrations on two truncated constructs corresponding to DBL1-3 and DLB1-4, respectively. The sample of the latter was of very good quality.

To compensate for the late start of the previous visit an additional shift was used in connection with MX measurements. The purpose was to confirm the measurements carried out at the previous visit on DBL1-4 and to investigate another truncated construct of VAR2CSA ID1-DBL4. Both samples gave satisfactory results.

Results obtained:

Scattering curves fit the expected statistics with respect to molecular weight estimated from the Bovine Serum Albumine standard kindly provided by the ESRF. In addition the statistics calculated from the inverse Fourier function fit the expected Mw and Volume for both VAR2CSA and for the truncated variants.
We finally succeeded in unambiguously identifying the positions of DBL domains 1, 5 and 6 in the VAR2CSA particle by measuring the scattering curves for strategically truncated variants of the VAR2CSA protein. The latest results truncated versions of VAR2CSA and ID1-4 have led us able to propose a model for the arrangement of domains within the VAR2CSA shape density, which is in agreement with the immunological data. A manuscript is in preparation describing these results.

We would like to conclude this report by expressing our great satisfaction with the performance of ID14-3. In the course of our experiments it has been constantly improved, sample changer, detector etc.
It has become an outstanding protein solution SAXS beamline (the best in the world!!) supported by an equally superb staff.