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:** Determination of the quarternary structure of key proteins involved in *Plasmodium falciparum* pathogenesis	**Experiment number**: MX1073
Beamline: ID14-3	**Date of experiment**: from: 26/10/2009 09:30 to: 27/10/2009 08:00	**Date of report**:
Shifts: 3	**Local contact(s)**: Mats Ökvist	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Stig Christoffersen, University of Copenhagen

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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:
It was the experimentalists first SAXS experiment at ID14-3, so the experiment also served as an introduction to the beamline, which was given in an excellent way by the local contact and one of the beamline responsibles Adam Round. Unfortunately the beamline was slightly misaligned, so time was spent to get it working optimally. Much time was spent on the BSA standard measurements as we made the mistake to initially use our own less pure Bovine Serum Albumin (BSA) and not the one provided by the ESRF. A lysozyme standard was also measured.
The protein that we managed to investigate in greatest detail is the PfEMP1 protein VAR2 that has been shown to be connected to pregnancy-associated malaria. The 310 kDa VAR2 protein is selectively transcribed in all placental parasites investigated to day. Its action is believed to be through binding to the placental receptor chondroitin sulphate A (CSA).
SAXS measurements were performed for three different concentrations of VAR2.
It is through the interaction with placental CSA that VAR2 exerts its action. Measurements were therefore also carried out for CSA and VAR2 in the presence of CSA.
It was also attempted to perform experiments on VAR2 labelled with the socalled nanogold, a 1.4 nm gold particle engineered to bind and localize histidine tagged proteins. Attempts adding the nanogold particle-solution to VAR2 protein solutions sadly resulted in precipitation of VAR2.

Measurements were also performed for VAR3 without its N-terminal domain (molecular mass 7kDa), which is one of the smallest PfEMP1 proteins. The molecular mass of VAR3 is around 80 kDa. Time was spent finding the right concentration range for the SAXS measurements of VAR3. Those performed at the initial higher concentrations showed clear signs of aggregation. Going to lower concentrations a series of measurements were succesfully conducted. Three concentrations of VAR3 was measured, as well as VAR3 in the presence of Nano-gold.

Unfortunately we did not notice that a misalignment of the beamline had occurred at the end of the 2nd shift just after our first meassurements on VAR2. This had an unfortunate impact on the subsequent measurements containing fewer counts and hence more noise. As a result the initial measurements on VAR2 were found to represent the most succesful results from this experiment.

Results obtained:

The measurements for VAR2 at different concentrations show the same overall shape for VAR2. However the molecular mass estimated by the SAXS measurement is almost twice its actual molecular mass. We are currently analyzing these data, but as there are problems with polydispersity effort has been put to improve the sample quality for subsequent measurements.

The effect on the structure of VAR2 upon binding CSA was investigated, but as a result of the nature of the CSA sample and the poorer signal/noise ratio caused by misalignment of the beamline, these studies are currently inconclusive.

VAR3 data are currently being analyzed. The data measured on VAR3 in the prescence of Nano-gold, show that presumably several VAR3 moieties are bound to each Nano-gold particle, which could explain the precipitation observed by the addition of nano-gold to VAR2.