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.

ESRF	**Experiment title:** Solution scattering of nuclear transport complexes.	**Experiment number**: MX-970
Beamline: ID14-3	**Date of experiment**: from: 27/7/09 to: 28/7/09	**Date of report**: 2/3/10
Shifts: 3	**Local contact(s)**: Adam Round	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The aim of our experiment carried out on 27-28th July 2009 was to obtain good quality SAXS data on various complexes of the nuclear protein export carrier Xpo1p (*S. cerevisiae*) to examine the conformational changes this protein may undergo upon binding of partner proteins. This 125kDa protein mediates nuclear export of proteins (such as HIV Rev) via binding of a leucine-rich nuclear export signal (NES), and also of small hnRNAs using adaptors [1,2,3]. The final export complex is comprised of Xpo1, its cargo, and the small GTPase Ran.

Complexes of Xpo1p and Yrb2p (the yeast homologue of RanBP3) were prepared for SAXS analysis using both full-length Yrb2p and its FG-repeat region alone. This protein has been shown to bind directly to Xpo1p and may play a role in stabilising the export complex by increasing the affinity of Xpo1p for its NES cargoes [4]. Analysis of previous SAXS data collected at ID14-3 from the full length Yrb2p:Xpo1p complex indicated some aggregation in the sample. Unfortunately, the new data collected during this experiment for both Yrb2p complexes appear also to suffer from the influence of non-homogeneity in the sample and this has prevented detailed interpretation of the solution structure of the complexes.

Mutants of Xpo1p were examined in their unbound form to collect data on whether changes to key residues within the molecule might alter the global conformation of the protein. These mutants included deletion constructs of a long loop between HEAT repeats 8B and 9A, and the C-terminal helix of the molecule. The loop deletion mutant shares very similar solution scattering to wild type Xpo1p. This finding suggests that this loop, which varies significantly in homologues of Xpo1p does not appear to play a significant role in regulation of the global conformation of the protein. SAXS data collected on the C-terminal helix deletion mutant of Xpo1p indicated the presence of aggregated species within the sample which has so far prevented interpretation of the effect of this mutation on the conformation of Xpo1p.

[1]T.Guan *et al.* (2000). Nup50, a nucleoplasmically oriented nucleoporin with a role in nuclear protein export. *Mol. Cell. Biol.* **20**:5619-5630.
[2]M.Fornerod *et al.* (1997) The human homologue of yeast CRM1 is in a dynamic subcomplex with CAN/Nup214 and a novel nuclear pore component Nup88. *EMBO J.* **16**:807-816
[3]D.Engelsma *et al* . (2004). Supraphysiological nuclear export signals bind CRM1 independently of RanGTP and arrest at Nup358. *EMBO J.* **23:**3643-3652.
[4]L.Englmeier *et al.* (2001) RanBP3 influences interactions between CRM1 and its nuclear protein export substrates. *EMBO Rep.* **2(10)**:926-932.