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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: Solution Scattering of Nuclear Export Complexes	**Experiment number**: MX-843
Beamline: ID14-3	**Date of experiment**: from: 17/3/09 to: 18/3/09	**Date of report**: 31/3/09
Shifts: 3	**Local contact(s)**: Adam Round, Petra Pernot	*Received at ESRF:*

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Report:

The aim of our experiment carried out on 17-18 March, 2009 was to obtain good quality SAXS data on the nuclear protein export carrier Xpo1p (*S.cerevisiae*). 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.

The current structural information available for Xpo1 is limited to a 2.3Å crystal structure of the C-terminal third of the human version of Xpo1 known as CRM1 [4], a SAXS structure of the full length unbound *S. cereviseae* Xpo1 protein [5], and a 22 Å electron microscopy reconstruction of the unbound human CRM1 [4]. These structures have not revealed the mechanism by which CRM1/Xpo1 is able to bind NES signals, nor the predicted conformational change of CRM1/Xpo1 upon substrate binding.

Karyopherin proteins involved in nuclear transport are thought to possess a high level of intrinsic flexibility. It has been suggested that CRM1/Xpo1 may adopt "open" and "closed" conformations analogous to Cse1 (the export protein for importin alpha) [6] and that a series of conserved residues which line the inner concave face of the central region of CRM1/Xpo1 may be involved in NES binding. It is thought that binding of substrates stores energy within the spring-like structure of karyopherins that is released once the transport step is complete.

During our experiment SAXS data was collected on Xpo1p alone, Xpo1p/S1 peptide, and Xpo1/Yrb2p. The S1 peptide is a NES peptide that has been shown to bind Xpo1 in the absence of RanGTP [7]. Yrb2p (the homologue of RanBP3 in humans) has been shown to bind directly to Xpo1p and may play a role in stabilising the export complex by increasing the affinity of Xpo1 for its NES cargoes [8].

Preliminary analysis of our data using the ATSAS software suite [9] has revealed a distinct change in the scattering of Xpo1 between the unbound and S1 peptide complex samples. The initial *ab initio* models

suggest that in its unbound state Xpo1 assumes a circular, closed conformation in close agreement with the EM model [4]. Upon binding of the S1 peptide, the second half of Xpo1 appears to twist away from the circular plane, and a small protrusion appears in the first half of the molecule. This may represent the loop predicted to exist in HEAT repeat 8 of Xpo1 which is thought to change conformation upon binding of cargo [4].

Data from the Xpo1/Yrb2p complex has so far been analysed to a lesser degree than the two other samples. The first models of this complex indicate an elongated arrangement of the proteins, with Yrb2p seeming to stack on top of Xpo1. The conformation of Xpo1 cannot yet be clearly delineated from the preliminary models. Further processing of the data and a greater number of *ab initio* analyses are required before conclusions as to the mode of Yrb2p binding to Xpo1 can be made.

[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]C.Petosa *et al*. (2004). Architecture of CRM1/exportin1 suggests how cooperativity is achieved during formation of a nuclear export complex. *Mol. Cell*, **16**:761-775**.**

[5]N. Fukuhara *et al* (2004) Conformational Variability of Nucleo-cytoplasmic Transport Factors. *The Journal of Biological Chemistry* **279**(3): p. 2176-2181.

[6] A.Cook *et al*. (2005). The structure of the nuclear export receptor Cse1 in its cytosolic state reveals a closed conformation incompatible with cargo binding. *Mol. Cell,* **18**:355-367.

[7] D.Engelsma *et al* . (2004). Supraphysiological nuclear export signals bind CRM1 independently of RanGTP and arrest at Nup358. *EMBO J.* **23**:3643-3652.

[8] L.Englmeier *et al.* (2001) RanBP3 influences interactions between CRM1 and its nuclear protein export substrates. *EMBO Rep.* **2(10)**:926-932.

[9]D. Svergun (1999). Restoring low resolution structure of biological macromolecules from solution scattering using simulated annealing. *Biophys. J*. 76: 2879.