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 via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can 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:** Time-resolved structural rearrangements of translocating ribosome	**Experiment number:** LS 2406
Beamline: ID 02	**Date of experiment:** from: 30.04.2015 to: 03.05.2015	**Date of report:**
Shifts: 9	**Local contact(s):** Dr. Gudrun Lotze, Dr. Theyencheri Narayanan	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The goal of this experiment was to investigate kinetics of structural rearrangements of the ribosomal complex in course of translocation by means of the time-resolved Small-Angle X-ray scattering (SAXS) analysis.

We have used highly purified, homogeneous and biochemically characterized ribosomal complexes, including: vacant 70S ribosomes from E. coli; functional initiation complex (carrying single fMet-tRNAfMet in the P site); functional pre-translocation complex (carrying deacylated tRNA in the P site and peptydil-tRNA in the A site); functional post-translocation complex (carrying peptydil-tRNA in the P site). We have performed:

I. Series of "static" measurements, using flow-through capillary cell setup.

We have measured extensive set of ribosomal complexes in initial and final conditions of forward and reverse translocation reactions, including various buffer conditions, the presence of trnasport RNAs, protein factors and low molecular weight ligands. The data

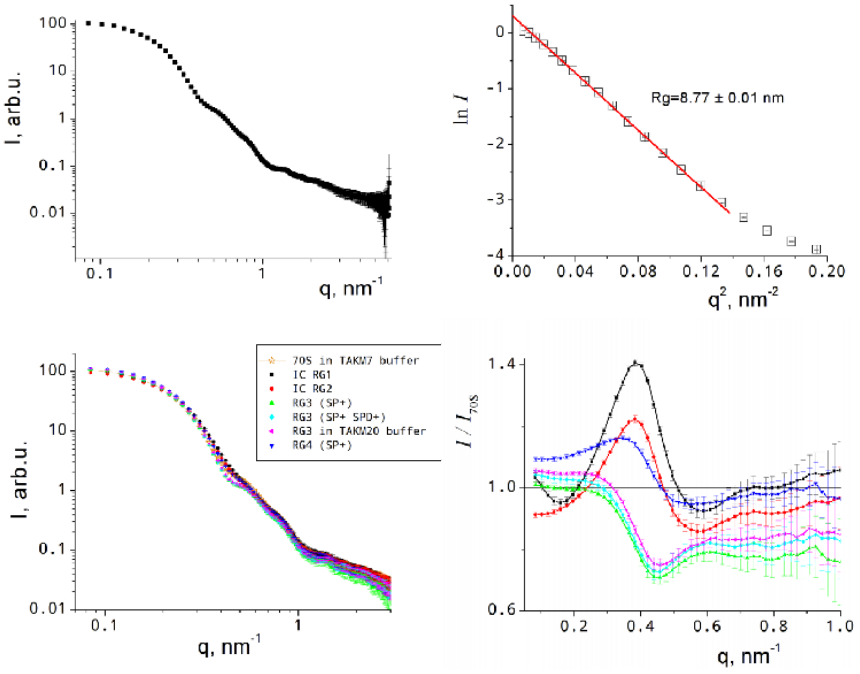

Figure 1. (top) SAXS from the 0.5 μM solution of 70S E.coli ribosome and the corresponding Guinier plot; (bottom) Comparison of the SAXS from initiation complexes (IC RG1 and IC RG2), pre-translocation complex (RG3) in different buffers, and post-translocation complex (RG4).

(typical SAXS curve for the samples containing 70S ribosome particles is shown in Fig.1, top) were well described by the model of monodisperse particles. The gyration radius of the particles varied between 8 and 8.8 nm, which is close to expected for the native *E.coli* ribosomes. No sign of aggregation was observed in the range of concentration between 0.2 and 1 μM. The concentration of 0.5 μM, where only the minimal interparticle interference was observed in the chosen range of momentum transfer, was used for most of the measurements. No radiation damage was observed, judsing by practically no difference in SAXS spectra taken with the exposure time varied by a factor of 3.

For the different ribosomal complexes we observed a significant variation in both the shape of the scattering curve, particularly in the range between 0.25 - 0.7 nm^{-1} and the size (gyration radius) of the particle (Fig.1, bottom). There are slight differences between initiation complexes programmed with regular mRNA (IC RG1) and mRNA with secondary structure elements (IC RG2), located downstream to codon, exposed in the A site, suggesting that mRNA structure can influence the global conformation of the ribosome.

Pre-translocation complexes (RG3) spectra demonstrate significant deviation in q range 0.25 - 0.55 nm^{-1} from vacant ribosomes, initiation and post-translocation complexes, indicating the global structural rearrangements of ribosomal particles. This observation supports the evidence provided by X-ray crystallographic and cryo-EM studies of ribosomal comlexes.

II. Series of "kinetic" measurements, using stopped-flow cell setup.

In this setup we have initiated a number of different reactions by mixing the solution of ribosomal complex with various components, including specialized protein elongation factor EF-G with different guanine nucleotides, deacylated tRNAs, specific inhibitors of translocation, or buffer. In all of the kinetic experiments we have observed only slight changes in the SAXS spectra, usually within 0.5% of the intensity (see Fig.1 top for a typical result of the kinetics series). The data was further analyzed using singular value decomposition algorithm that yielded 2 - 3 principal components of SAXS spectra with the singular values significantly above the noise level (Fig.2, middle plot). This approach allowed us to pinpoint the small variations in SAXS that reflect the structural changes associated with the reaction of translocation and measure their kinetics (Fig.2, bottom).

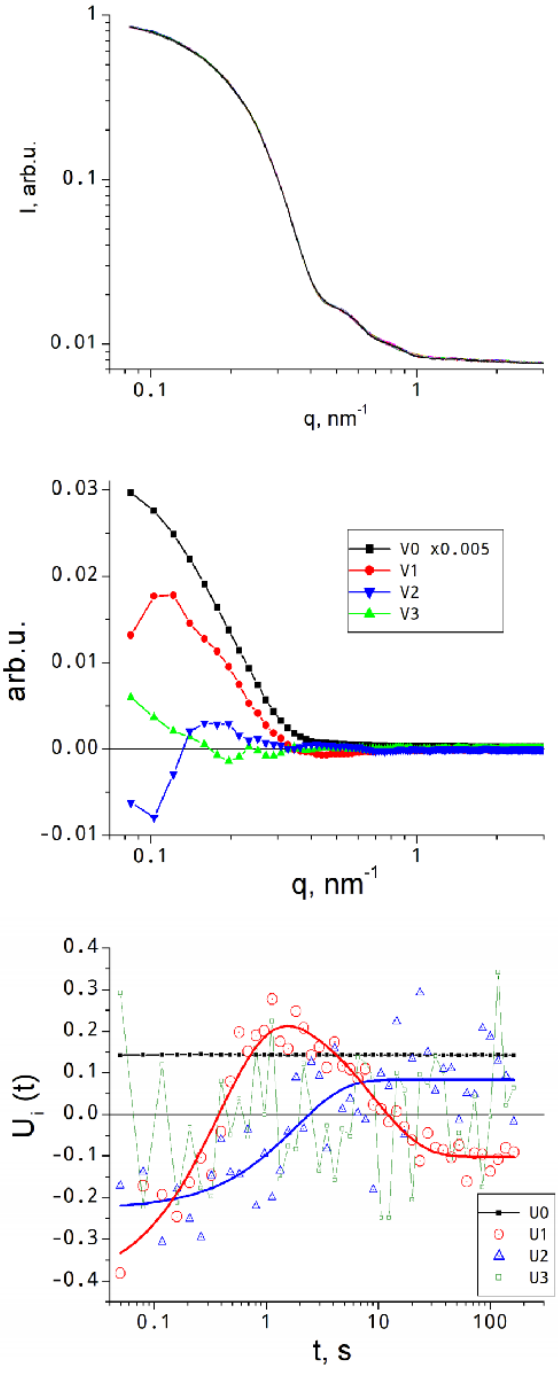

Figure 2. Series of 50 SAXS from pre-translocation complex upon mixing with 1 μM EF-G in the presence of 0.5 mM GTP (top), four principle components of the spectra yielded by SVD (middle) and the kinetics of these components fitted to exponential functions (bottom)