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:** RHDV-HBGA interaction	**Experiment number**: MX1660
Beamline: ID 23-1	**Date of experiment**: from: 03.09.14 to: 04.09.14	**Date of report**: 22.10.14 (update)
Shifts: 2	**Local contact(s)**: Popov, A.	*Received at ESRF:*
Names and affiliations of applicants (* indicates experimentalists): Dr. Grant Hansman		

Report:

Crystals for the P domain of the rabbit hemorrhagic disease virus (RHDV) variant N11 in complex with the HBGAs Lewis Y tetrasaccharide and H2 trisaccharide, as well as a synthetic compound (called com78) were collected at beamline ID23-1. Two single crystals of the N11-H2 complex diffracted up to 0.94 Å and 0.97 Å, datatsets were collected. For the N11-LeY complex three datatsets were collected from two single crystals that diffracted to 0.96 and 1.2 Å . Three datasets were collected from three single crystals of the N11-com78 complex that diffratced in the range of 1.2 Å to 2.4 Å . In total, 11 datatsets were collected for this experiment, from 10 single crystals that all diffracted very well. Molecular replacement of all datatsets was performed in phenix using the autoprocessed EDNA files with an unpublished model of the unliganded N11 P domain (MX1560). Poor electron density for the fucose moitey of H2 was visible. The other two ligands didn't show clear patches of electron density. The solved complex structures of N11-H2 and N11-LeY were used to confirm previous results (MX1560) to finish the data for submission of a manuscript. More data on N11-HBGA binding from crystallography will aid a deeper understanding of the binding mechanism. Since several features were found similar to the human norovirus binding site (manuscript in review), potential entry inhibitors (like com78) that bind to human norvirus P domains were and still have to be tested.

ESRF	**Experiment title:** Astrovirus-HBGA interaction	**Experiment number**: MX1660
Beamline: ID 23-1	**Date of experiment**: from: 03.09.14 to: 04.09.14	**Date of report**: 22.10.14 (update)
Shifts: 2	**Local contact(s)**: Popov, A.	*Received at ESRF:*
Names and affiliations of applicants (* indicates experimentalists): Dr. Grant Hansman		

Report:

Several datasets of single crystals of an astrovirus serotype 8 capsid protein in complex with HBGAs were collected at ID23-1. A recent report indicated that astroviruses might be able to bind HBGAs in a similar way to human norviruses. Therefore, the projection domain of the astrovirus coat protein was co-crystallized with a common set of HBGAs, namely H2 trisaccharide, A trisaccharide, B trisaccharide, Lewis A trisaccharide, and Lewis Y tetrasaccharide, as well as sialic acid and N-glycolylneuraminic acid. 34 single crystals were taken to the beamline. Due to limitations of beam time datasets of 20 crystals of Astro-complexes (triplicates or quadruplicates of Astro-H2, Astro-Atri, Astro-sialic, Astro-LeY, Astro-LeA) were collected that all diffracted well in the range of 1.7 Å to 2.3 Å. Processing of all collected datatsets was performed with xds. Molecular replacement was done using a model available from a recent publication (pdb 3QSQ).

The spacegroup that was initally calculated from EDNA and xds (C222) didn't give a usable MR result. Changing the spacegroup to P121 showed a tetramer in the assymetric unit that fitted well the electron density. No additional electron density was found for any of the investigated carbohydrates. Since not all complex crystals could be collected, it cannot be inferred from the present data that astrovirus doesn't bind HBGAs by crystallography. More studies are needed to characterize the binding behaviour more detailed.

ESRF	**Experiment title:** **Crystal structure of RNA polymerase of human norovirus GII.4 with ligands**	**Experiment number**: MX1660
Beamline: ID 23-1	**Date of experiment**: from: 03.09.14 to: 04.09.14	**Date of report**: 22.10.14 (update)
Shifts: 2	**Local contact(s)**: Popov, A.	*Received at ESRF:*
Names and affiliations of applicants (* indicates experimentalists): Dr. Grant Hansman		

Report:

The objective of this study is to gain the understanding of the interactions of the RNA polymerase of a GII.4 norovirus with RNA primer and dNTPs. Furthermore, we also want to examine the binding of norovirus polymerase with known RNA polymerase inhibitors. To initiate with this aim, recombinantly produced RNA polymerase of GII.4 strain from 2012 outbreak (NSW) was soaked with three different inhibitors (434, 222, 563) as well as dNTPs and RNA primer for different time spans. Diffraction-data were collected from two crystals soaked with the drug 563 at 1.78 Å and 1.6 Å, and one crystal soaked with RNA primer at 1.96 Å. All crystals were in space group C121. In order to check for bound ligands, the map files generated from the data processing by EDNA were used for molecular replacement and model building. Molecular replacement was performed in phenix using the apo-structure of NSW polymerase as a template. We have recently solved this apo-structure of NSW polymerase (unpublished). Phenix.refine was used for refinement and COOT for model building. No bound ligands were observed in these crystal structures. The next strategy would be co-crystallization of polymerase with the ligands. We also have a new construct of full-length NSW polymerase.

ESRF	**Experiment title:** **Crystal structures of nano-85 in complex with GII.4 P domains**	**Experiment number**: MX1660
Beamline: ID 23-1	**Date of experiment**: from: 03.09.14 to: 04.09.14	**Date of report**: 22.10.14 (update)
Shifts: 2	**Local contact(s)**: Popov, A.	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Grant Hansman

Report:

Previously, we solved a structure of a nanobody (Nano-85) in complex with a GII.10 P domain using diffraction data obtained on ID23-1 (MX1596). According to our biochemical data Nano-85 is also able to bind other norovirus genotypes, including the most prevalent GII.4 group. To analyze the binding site of Nano-85 on the GII.4 P domain and to compare it to Nano-85-GII.10 P domain complex we purified complexes of Nano-85 bound to two different GII.4 P domains. Both complexes were crystallized and multiple crystals were obtained after 2 weeks. Out of two crystals of a Nano-85-Saga P domain complex one diffracted to 2 Å, whereas the other showed no diffraction. Datasets for four crystals of Nano-85-Saga P domain were collected with resolution up to 1.9 Å. Data processing was performed using EDNA autoprocessing files in space group P212121 and P22121 for Nano-85-NSW and Nano-85-Saga P domain, respectively. Molecular replacement was done using the structure of the Nano-85–GII.10 P domain complex (MX1596). In all three structures Nano-85 bound in an identical manner, confirming cross reactivity of Nano-85 with various norovirus strains on a structural level. Furthermore, data obtained during this session allowed us to start the patenting process of Nano-85.

ESRF	**Experiment title:** **Crystal structure of Fab with GII.4 P-domain**	**Experiment number**: MX1660
Beamline: ID 23-1	**Date of experiment**: from: 03.09.14 to: 04.09.14	**Date of report**: 22.10.14 (update)
Shifts: 2	**Local contact(s)**: Popov, A.	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Grant Hansman

Report:

Currently, several monoclonal antibodies that are able to detect human noroviruses are available. However, there is only one structure of a broadly reactive monoclonal antibody (mAb) fragment in complex with a GII.10 norovirus P domain published, and none for strain specific antibodies. Thus, the purpose of this project was to identify the binding site of an anti-GII.4 mAb on the GII.4 norovirus P domain. Fab#7 fragment from a commercially available mAb was purified and used to form a complex with GII.4 NSW P domain. During the MX1660 session, we screened 8 crystals, out of which one diffracted up to 3 Å. Multiple datasets for this single crystal were collected on ID23-1 and processed with XDS. Structure was solved with molecular replacement using the apo-structure of GII.4 P domain and PDB entry 25C8 as models. Preliminary structure of Fab#7–GII.4 P domain indicated that Fab bound to the region on the P domain that is involved in HBGA binding. Further data with higher resolution would give more information on exact manner of binding.

ESRF	**Experiment title:** **Crystallization of norovirus shell domain**	**Experiment number**: MX1660
Beamline: ID 23-1	**Date of experiment**: from: 03.09.14 to: 04.09.14	**Date of report**: 22.10.14 (update)
Shifts: 2	**Local contact(s)**: Popov, A.	*Received at ESRF:*
Names and affiliations of applicants (* indicates experimentalists): Grant Hansman		

Report:

The major structural protein of the norovirus capsid is composed of two domains: P domain and shell domain. Whereas the P domain is well studied and structures of various P domains are solved and available, there is still no structure known for the shell domain. Structural data of the shell domain are important for an understanding of the whole capsid organization. It may also shed light on binding of broadly reactive mAb to the shell domain. For this project we crystallized GII.10 and GII.4 shell domains in 10 different mother liquors and screened for diffraction. Only crystals obtained in conditions C8 (GII.10) and C9 (GII.4) diffracted to about 5 Å, whereas there was no diffraction observed for any other crystals. Further optimization of cryo-solutions and mother liquor composition is required to improve the resolution.