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*

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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.

<table>
<tr><td> </td><td colspan="2">Experiment title:
Defining the mechanism and timing of Toxoplasma invasion effectors using X-ray fluorescence imaging</td><td>Experiment number:
LS-2977</td></tr>
<tr><td>Beamline:</td><td colspan="2">Date of experiment:
from: 03/06/2021 to: 07/06/2021</td><td rowspan="2">Date of report:
12/01/2022
Received at ESRF:</td></tr>
<tr><td>Shifts:</td><td colspan="2">Local contact(s):
Peter Cloetens</td></tr>
<tr><td colspan="4">Names and affiliations of applicants (* indicates experimentalists):

Matthew W. Bowler*, EMBL, Grenoble, France
Mohamed-Ali Hakimi, IAB, Grenoble, France</td></tr>
</table>

Report:

Toxoplasma gondii is an intracellular parasite that infects over half the world's population and is the causative agent of toxoplasmosis – a condition extremely dangerous for developing foetuses and those with a weakened immune system (e.g. patients undergoing chemotherapy or infected with HIV). However, most people are unaware of infection as the organism tightly controls its host's immune response using a range of secreted effector proteins. A common strategy for intracellular pathogens is to take control of host signaling networks in order to re-wire the response to its own requirements (parisitism). A range of secreted agonists has recently been discovered in *Toxoplasma gondii.*

We study the timing, dynamics and transport of proteins delivered by *Toxoplasma* in human cells following invasion. This would normally be performed by labeling proteins genetically with a fluorescent marker such as GFP. This cannot be performed with exported *Toxoplasma* proteins as they are unable to cross the parasite-containing vacuole membrane. So far, all information has been gained by immunostaining using small tags (HA, FLAG). While informative, only limited information is gained on timing and location of proteins. We have labeled these GRA proteins with a short metal binding motif (12 histidine residues collating Ni^{2+}) that does not interfere with export and could be detected by XRF microscopy.

The beamtime allocated to LS2977 was used as a proof of principal experiment to demonstrate that a protein linked to a nickel ion can be traced using XRF microscopy. Additionally, the high resolution phase contrast imaging of *Toxoplasma* residing in an infected human cell, in combination with the distribution of elements in these cells, is of high scientific value.

A number of samples were tested, including several cells infected with the his tagged GRA16, uninfected cells exposed to the same concentration of nickel ions (control 1), and cells infected with wild type toxoplasma (control 2). Samples were plunge cooled before allocated beamtime.

The beamtime was very successful – several cells from both test and control groups were examined. This involved screening a large number of samples as not only did we have to search for cells we also had to search for cells that had been infected. After several tests we found that toxoplasma could be readily identified in low resolution XRF scans from their high potassium content. This improved location of infected cells.

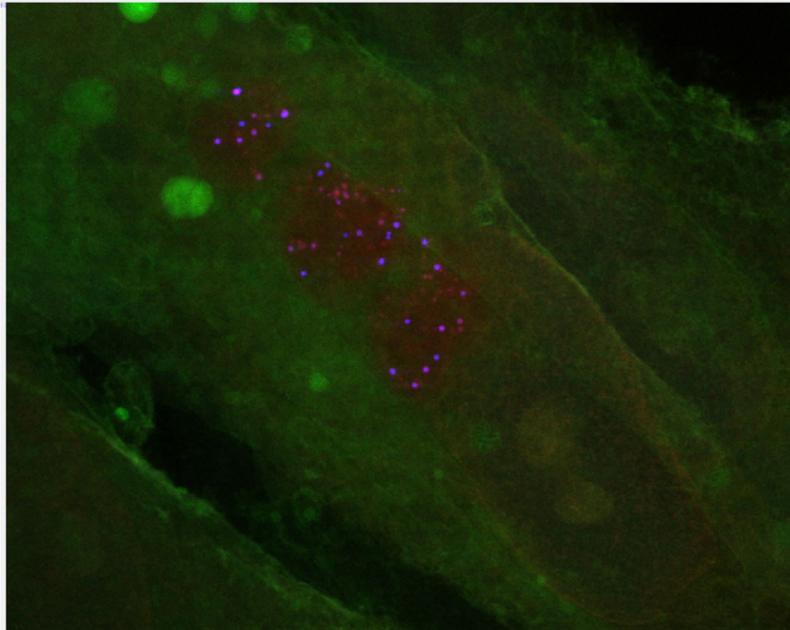

Figure 1. X-ray fluorescence microscopy of HFF cells 24 hours post infection by a *Toxoplasma* strain with tagged GRA16. The distribution of Potassium (red), phosphorous (blue) and nickel (green) is shown. The parasites can be seen depleted in nickel as they have not been exposed to the metal, with the bright spots being the dense granules that contain the GRA proteins.

An example of an XRF scan is shown in Figure 1. The XRF defines the cell structure and the nickel map has distinct features clearly showing the distribution of GRA16 within the cell. Of particular interest is the accumulation of GRA16 in the nucleolus – GRA16 interacts with USP7 – a protein that controls the levels of p53, a protein that determines if a cell goes into apoptosis, and is known to be prevalent in the nucleolus.

Main results:

1. The tagged GRA16 protein excreted from *T.gondii* can be seen in the host cell at high resolution in the Ni^{2+} spectra validating the concept of labelling proteins using a 12 histidine tag
2. GRA16 is located in the cytoplasm and nucleus of infected cells with particularly high concentrations in the nucleoli – this provides mechanistic insight into how *T.gondii* prevents cell death.
3. The distribution of elements in an infected cell is very different when compared to uninfected cells, this is particularly fascinating when looking at the Fe^{2+} spectra. As *T.gondii* is dependent on its host for iron we have gained insights into how the parasite controls lysosomes in the host cell
4. Phase contrast tomograms reveal the nature of Toxoplasma residing in human cells at 50 nm resolution

The measurements have produced a large volume of XRF and tomographic data that we are currently processing. The data have revealed many new features in the host-pathogen interaction and have demonstrated the use of a metal tag in XRF microscopy. These results are of high scientific value and results are currently being prepared for several publications. Additionally, we hope to include these data in multi-scale imaging experiments combining them with data of an X-ray crystallographic structure of GRA16 in complex with the human protein USP& that we have determined and also obtain sub-nm data using cryo-ET on areas of interest determined using XRF and tomography. We would like to thank Peter Cloetens for his incredible support before during and after these experiments and the whole ID16A team for an incredible instrument.