EUROPEAN SYNCHROTRON RADIATION FACILITY

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ESRF

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:** Mechanisms of quinoline-family drug action and drug resistance in the malaria parasite *Plasmodium falciparum*	**Experiment number**: LS-2704
Beamline:	**Date of experiment**: from: 16.02.2018 to: 20.02.2018	**Date of report**: 26.02.2018
Shifts: 12	**Local contact(s)**: Yang Yang	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Sergey Kapishnikov, University of Copenhagen, Denmark

Jens Als-Nielsen, University of Copenhagen, Denmark

Leslie Leiserowitz, Weizmann Institute of Science, Israel

Report:

The general purpose of this experiment was to investigate the interaction of the classical malaria drug Chloroquine with an infected red blood cell *in vivo*. As in previous experiments a snapshot of the *in-vivo* condition was reached by high pressure cryo-freezing of the hydrated infected red blood cell. We have shown in the past[1] that the combination of soft X-ray tomography and fluorescence imaging of the same red blood cell can enable a biochemistry analysis of the concentration of hemoglobin, potassium, and hemozoin crystals as well as the drug in different organelles of the cell such as the parasite digestive vacuole or its cytosol, and the cytosol of the red blood cell outside the parasite. The analysis requires scrutinizing care, and within the short period (less than two weeks) between the experiment and the deadline for submission of this report this has not been possible to accomplish. Nevertheless, we are convinced that the quality of the data obtained will enable us to do so in the near future.

There is however one important aspect of the experiment that we are able to report on, and that is the interaction between the drug and hemozoin (Hz) crystals. Detailed calculations[2,3] have indicated that Chloroquine will bind to specific faces of Hz crystals and thereby impede binding of heme dimers hence arresting growth of Hz crystals. As the result, free heme will accumulate in the digestive vacuole and kill the parasite. Although this model of drug action is generally favored by the research community, it has not been confirmed experimentally. Therefore, this was a primary motivation for the present experiment. Instead of Chloroquine (ClQ) we have used the isomorphous molecule Bromoquine (BrQ) because X-ray fluorescence

from Cl could be caused from other molecules or ions than ClQ. The K-edge energy for Br, about 12 keV, is conveniently lower than the exciting beam energy of 17.05 keV. In one infected red blood cell we observed an isolated Hz crystal, presumably escaped from the digestive vacuole by its rupture, and indeed with a high local concentration of Br around the faces of the crystal. The image data are shown in two panels in the figure below. The two panels show the fluorescence images of Fe atoms, bound to heme comprising Hz crystals, and Br atoms bound in the drug molecule BrQ. The figure shows unambiguously that the drug molecules have a pronounced affinity to bind to the crystal faces, and a scrutinizing analysis will reveal whether it corresponds to a sub-monolayer, a mono-layer (or more) of drug molecules on the surface of the Hz crystal. The image would be even more informative with the beam focus closer to its theoretical value of 12 nm [4] rather than the 30-50 nm achieved at 17.1 keV because with a 12 nm resolution one could have identified the (h,k,l)-Miller indices of the faces of the Hz crystal by its morphology and observed whether the BrQ binding depends on the face Miller indices as expected by the theoretical calculation alluded to above. This is important in a wider perspective, because if confirmed the reliability of the theoretical model would be enhanced considerably and would stimulate computation of modified drug molecules eventually leading to a better drug.

The discrepancy between the observed and the theoretical focal spot size is due to a manufacture imperfection in the KB optics available at 17.05 keV. It was recently shown by the beamline staff [4] that the KB optics available for 33.6 keV was indeed very close to being perfect with a resulting focal spot size of only 13 nm. We have therefore proposed a continuation of our experiment to be carried out with the 33.6 keV optics which will furthermore enable us to also investigate another promising drug of the quinoline family, namely RuQ.

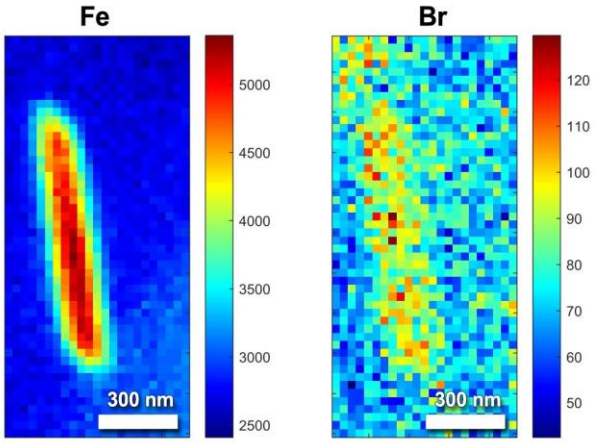

Figure 1 X-ray fluorescence maps from a bromoquine-affected hemozoin crystal.

References:

1 Kapishnikov, S., Leiserowitz, L., Yang, Y., Cloetens, P., Pereiro, E., Awamu Ndonglack, F., Matuschewski, K. & Als-Nielsen, J. *Scientific Reports* **7**, 802 (2017).

2 Buller, R., Peterson, M. L., Almarsson, O. & Leiserowitz, L. *Cryst Growth Des* **2**, 553-562 (2002).

3 Dubar, F., J.Egan, T., Pradines, B., Kuter, D., Ncokazi, K. K., Forge, D., Paul, J.-F., Pierrot, C., Kalamou, H., Khalife, J., Buisine, E., Rogier, C., Vezin, H., Forfar, I., Slomianny, C., Trivelli, X., Kapishnikov, S., Leiserowitz, L., Dive, D. & Biot, C. *ACS Chemical Biology* **6**, 275-287 (2011).

4 Cesar da Silva, J., Pacureanu, A., Yang, Y., Bohic, S., Morawe, C., Barrett, R. & Cloetens, P. *Optica* **4** (2017).