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Experiment Report Form

	Experiment title: Quantitative mapping of Fe concentration at nanoscale spatial resolution in frozen hydrated bacterial injured human cells	**Experiment number**: LS2433
Beamline: ID16A-NI	**Date of experiment**: from: 24th February 2016 to: 29th February 2016	**Date of report**: 17th March2016
Shifts: 15	**Local contact(s)**: Sylvain Bohic, Yang Yang	*Received at ESRF:*

Names and affiliations of applicants:

Gramaccioni Chiara, Physics Department University of Calabria, Cosenza (Italy), CNR-Nanotec, c/o Dept. of Physics Univiversity Sapienza, Roma, Italy

Lagomarsino Stefano, CNR-Nanotec, c/o Dept. of Physics Univiversity Sapienza, Roma, Italy

Berlutti Francesca, Dept. of Public Health and Infectious diseases, University Sapienza Rome (Italy)

Frioni Alessandra, Dept. of Public Health and Infectious diseases, University Sapienza Rome (Italy)

Peter Cloetens, European Synchrotron Radiation Facility (ESRF), 38000 Grenoble, France

Yang Yang, European Synchrotron Radiation Facility (ESRF), 38000 Grenoble, France

Report:

The main aim of this proposal LS 2433 was to quantitatively determine the intracellular map of iron concentration at nanoscale spatial resolution in cells stimulated by bacterial lipopolysaccharide (LPS) in the presence or absence of lactoferrin, (Lf), an iron-chelating glycoprotein of natural immunity (Valenti & Antonini 2005, Berlutti et al. 2011). We chosen the human macrophage THP1 cell line as macrophages are at the frontline of defence against bacterial pathogens able to modulate the intracellular iron concentration upon different external stimuli as LPS. We prepared four different THP1 preparations: untreated cells, cells treated with LPS or Lf, and cells treated with LPS plus Lf.

In this experiment, we used frozen hydrated cells, using the cryo stage at the ID16 NI beamline, in order to examine cells as close as possible to their native state, and to avoid radiation damage. In this case the illuminated volume cannot be measured with AFM, as we made in the experiment LS 2362 with freeze-dried cells, and therefore we planned to use nanotomography for this purpose. Unfortunately it was not possible to proceed with the tomography due to unexpected technical problems, but we succeeded in recording fluorescence maps and 2D phase contrast images. The frozen hydrated cells were very intact, confirming the

suitability of the sample preparation procedures. Frozen hydrated cells were prepared and controlled in our laboratory, transported to ESRF and measured on the ID16NI beamline under cryogenic conditions. We didn't analyze all four different macrophage preparations because of limited time, first commissioning of cryo-conditions and precharacterization of samples to be optimized. From the data acquired during the experiment we plan to derive the weight fraction spatial distribution, however in order to obtain the concentration maps, we would need to perform tomographic scans; and it was for this reasons that we have applied another proposal (LS 2551) in order to complete the experiment, by acquiring tomographic scans together with the fluorescence maps, in order to derive also concentration maps, the quantity of reference in the biological process. Nonetheless, we find that the data acquired in the last experiment LS 2362 is extremely interesting and at the fore-front of research on single cells.

With the experiment we expext to obtain useful information about the role of Lf in iron homeostasis in LPS-stimulated cells.

The assistance from the staff was excellent. We already started the analysis of the data, and and we hope to obtain useful results quite soon.

References

[1] Malucelli E. et al., 2014. Anal. Chem, 86 5108-5115.
Valenti & Antonini, Cell Mol Life Sci 62(22):2576-87, 2005
Berlutti et al. Molecules. 2011;16(8):6992-7018.