ESRF	**Experiment title:** Effect of protein lipidation on the structural properties of exosome vesicles released by skeletal muscle cells	**Experiment number**: MX-1765
Beamline: BM29	**Date of experiment**: from: 13/12/2015 to: 14/12/2015	**Date of report**: 26/08/2016
Shifts: 3	**Local contact(s)**: Martha Brennich	*Received at ESRF:*

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Report:

The general purpose of experiment MX-1765 was to investigate the structure of extracellular membrane vesicles (exosomes) and to determine the experimental conditions and feasibility to model the electronic density profile in such a complex system. Therefore we performed a series of measurement of exosomes obtained from different cell lines and with different treatments, as specified in the caption of Fig. 1. While the modelling of these curves will require a challenging effort, we notice the remarkable qualitative peculiarity of exosomes extracted from vegetal cells (lemon), as expected from our recent results (Raimondo et al. 2015 Oncotarget 6:19514-19527).

The more specific aim was to study and identify any structural difference in vesicles released by muscle cells in relation to a different extent of protein lipidation, which was shown to regulate membrane biogenesis (Romancino et al. 2015 FEBS Lett. 587:1379-1384). Therefore we extracted exosomes from muscle cell treated with an inhibitor of protein palmitoylation (EX-2BP) and compared them with exosomes extracted from untreated cells (EX-CTR). Larger size micro vesicles were also extracted from the same 2BP treated (MV-2BP) and untreated (MV-CTR) cellular lines. The size and shape of vesicles were previously controlled on the same preparation by Dinamic Light Scattering, performed at our home laboratory, and by Atomic Force Microscopy, performed at the PSCM facility of the ESRF. The scattering intensities measured in direct injection experiments show a similar pattern with a more structured character in the case of smaller exosome with respect to micro vesicles (Fig. 2).

Since a main difficulty is given by vesicles heterogeneity, we made an attempt to use HPLC separation for some type of vesicles. However the initial concentration (estimated as 1mg/ml in total protein content) was too low to allow a reliable signal to noise ratio.

The remaining time was dedicated to other systems of our interest, two serpin proteins (Noto et al. 2015 Sci. Rep. 5:13666): C1-Inhibitor (C1I) and Neuroserpin (NS). C1I was measured in physiological buffer at

different concentrations. The form factor was determined at low concentration (with Rg=5.75 nm) both by direct injection and by measuring the elution peak from HPLC column. The I(q) was factorised to determine the structure factor in the curves at high concentrations (Fig. 3). We notice the prevalence of a repulsive contribution (likely due to electric charges) and at the same time a peak around 0.6 nm-1, related to a small range attractive contribution.

The last measurement was done upon HPLC separation of a specific protein complex: a homodimer made by two monomers of the neuroserpin N157C mutant linked by the induced sulfur bridge. The measure was successfully fit by SASREF (Fig. 4).

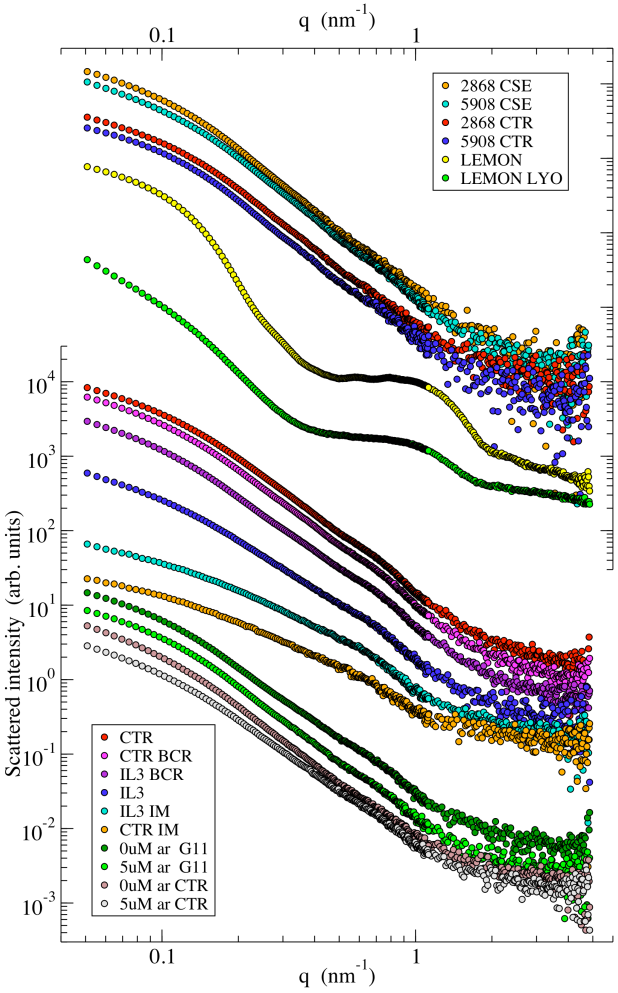

Figure 1 – Intensity of X-rays scattered by vesicles from different cell lines, as in the legend.
[1] Lung cancer model cell lines (2868 CTR, 5908 CTR), and treated with Cigarette Smoke Extract (2868 CSE, 5908 CSE).
[2] Lemon juice (LEMON); lyophilised and re-suspended vesicles from lemon juice (LEMON LYO).
[3] HEK293 cell lines (CTR), loaded with BCR/siRNA (CTR BCR) or with Imanitib drug (CTR IM); equivalent vesicles functionalised with IL3 receptors (IL3, IL3 BCR, IL3 IM).
[4] HEK293 or G11 cell lines loaded with different doses of retinoic acid (0, 0.1 5 uM ar CTR; 0, 0.1, 5 uM G11).

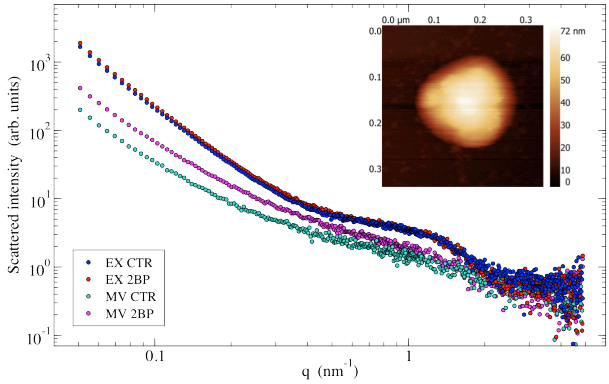

Figure 2 – Intensity of X-rays scattered by vesicles from muscle cells. Inset: AFM image of EX-CTR sample.

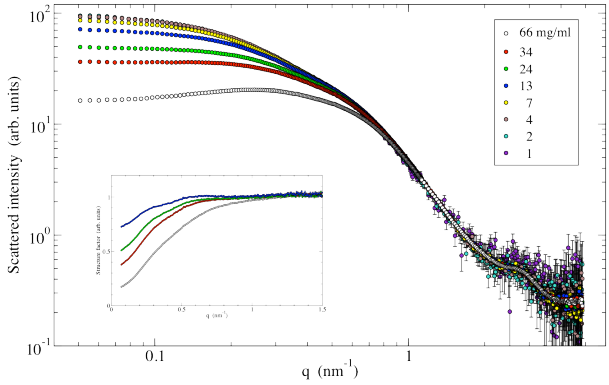

Figure 3 – Intensity of X-rays scattered by C1-Inihibitor at different concentrations. Inset: Structure factors.

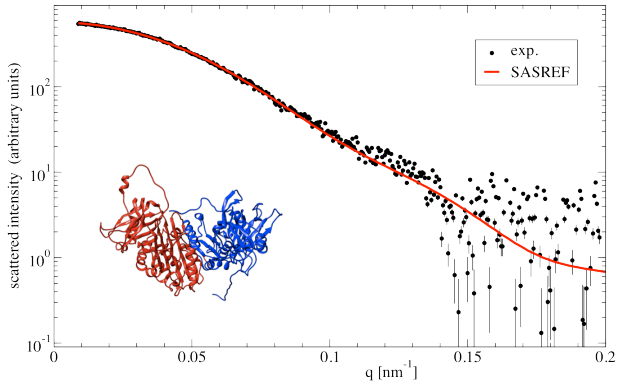

Figure 4 – Intensity of X-rays scattered by dimers of N157C neuroserpin after SEC separation. Inset: Model obtained by fitting experimental data by SASREF.