ESRF	**Experiment title:** µFTIR and nXRF study of the effect of DG4-His-Mal dendrimer encapsulated in liposomes	**Experiment number**: LS3075
Beamline: ID21	**Date of experiment**: from: 8 June 2022 to: 13 June 2022	**Date of report**: 20-9-2022
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Report:

Alzheimer Disease (AD) is a neurodegenerative process distinct from normal ageing by the presence of Senile Plaques composed of Abeta peptide. Dendrimers have been shown to possess properties as antiamyloidogenic agents. Maltose modified dendrimers decorated with histidine's (DG4-His-Mal) are nanoparticles that prevent APP/PS1 transgenic mice from developing cognitive deficit and µFTIR studies have shown that DG4-His-Mal reduce the formation of early aggregates and fibrils in the cortex. To improve this effect, we have administered DG4-His-Mal encapsulated in liposomes to 5xFAD transgenic mice (model for AD). The experiment is still on going, however, the midterm cognitive test show that DG4-His-Mal and liposomes alone improve the performance in cognitive test and have a synergic effect when administered together in AD transgenic mice. To understand the effects of these treatments we combine histochemical analysis, with µFTIR (amyloid structure) and XRF (metal distribution).

Samples measured:

- Brain (hypocampus) slices from 4 5xFAD transgenic mice. Each sample x2.
- Brain (hypocampus) slices from 5 Wild Type (WT) animals. Each sample x2.
- Brain (hypocampus) slices from 5 5xFAD transgenic mice treated with His-Mal-dendrimer encapsulated in liposomes.

All these samples have been previously measured using microFTIR at ALBA for the localization of amyloid plaques.
At ID21 Fe X-ray fluorescence was measured in the areas where the plaques were located. XANES spectra were also acquired in points within and outside the amyloid plaques.

The results from experiment LS3075 carried out at ID21 beamline using X-ray fluorescence, when put together with previou infrared imaging experiments carried out at ALBA, show that amyloid plaques in Alzheimer's transgenic mice are more oxidized than wild type mice brain tissue and accumulate metals. We have also detected a decrease of lipid oxidation and in the accumulation of metals for the animals treated with dendrimer encapsulated in liposomes making this system very promising for the treatment of Alzheimer disease (Fig 1). The study of metals was focused on the study of Fe and K distribution (measured at ID21-ESRF) and we observed that 5x FAD transgenic mice accumulates K and Fe in plaques and, interestingly, 5x FAD transgenic mice treated with DG4-His-Mal encapsulated in liposomes showed lower levels of these metals (Fig 1C).

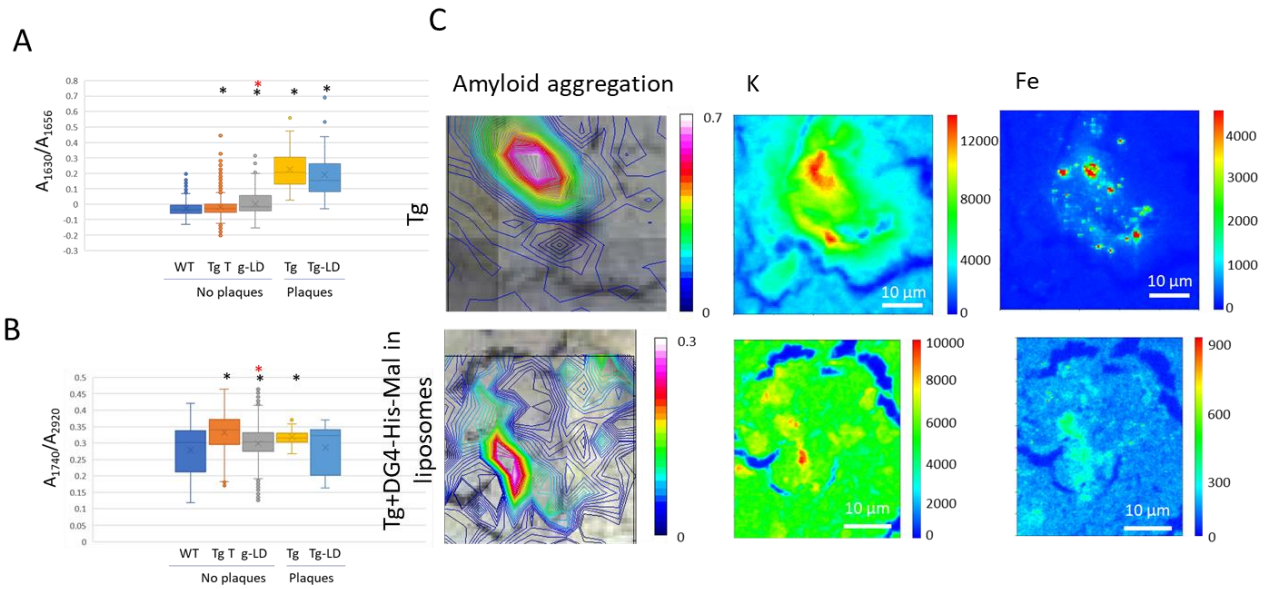

Figure 1. A) Box plot of Amyloid aggregation ratio (A_{1630}/A_{1656}) measured using microFTIR at ALBA, B) box plot lipid oxidation ratio (A_{1740}/A_{2920}) measured using microFTIR at ALBA. Oxidation ratio was determined around and at the detected plaques. Black asterisks show that data is statistically different from WT (ttest p≤0.05) and the red asterisks show that Transgenic(Tg) and transgenic mice treated with encapsulated dendrimer (Tg-LD) results difference is significative (ttest p≤0.05). C) Amyloid aggregation distribution (amyloid plaque,A_{1630}/A_{1656}) of a transgenic mice plaque and a treated with DG4-His-Mal dendrimer encapsulated in liposomes and K amd Fe X-ray fluorescence corresponing to the plaques detected by infrared.

The analysis of the XANES sprecta measured in all samples both within and ouside the amyloid plaques did not yield any apparent different populations. These results show therefore that no differences in the os¡xidation state of Fe were detected in the study.

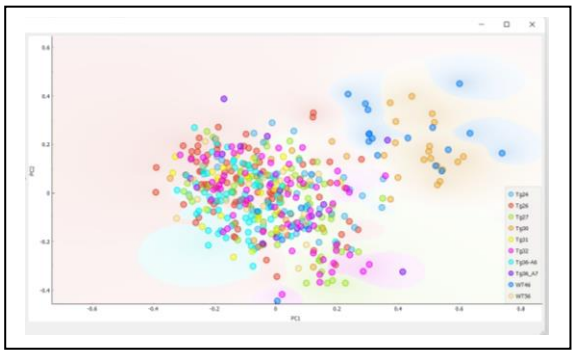

Fig. 2. PCA analysis of the Xanes spectra measured in all the samples (WT, transgenic and treated transgenic mice) measured both within and outside the amyloid plaques.

We have previously published that human amyloid plaques also show higher levels of lipid oxidation [Benseny-Cases *et a*l. (2014) Analytical Chemistry 86 (24), 12047-12054] and that human amyloid plaques accumulate metals [Álvarez-Marimon et al. (2021) ACS Chem Neurosc., 12, 11, 1961–1971]. Our results with the this triple transgenic mice model are consistent with the results from the human samples and contribute to validate the animal model for this type of studies.

We have also detected a decrease of lipid oxidation and in the accumulation of metals for the animals treated with dendrimer encapsulated in liposomes (panel 1C) making the DG4-His-Mal dendrimer encapsulated in liposomes as a delivery nano-vehicle a very promising system for the treatment of Alzheimer disease. Since the animals analysed have a better performance in a cognitive test, this data is especially interesting for the study of the molecular events (protein accumulation, lipid oxidation, metal accumulation), indicating that the use of this synchrotron-based techniqhes is adequate in order to help undertanding the mechanisms that at a molecular level can explain the benefict of the treatment with dendrimers.

Since there are differences Fe content in treated and non-treated animals, we are now considering exploring other metals that can be relevant to the disease such as Cu and Zn.