ESRF	**Experiment title:** MicroFTIR and microXRF characterization of dense and diffuse plaques from Alzheimer Disease affected brains: Secondary structure, oxidation and metal content determination	**Experiment number**: LS-2638
Beamline: ID21 and ID16B-NA	**Date of experiment**: from: 04/03/2017 to: 08/03/2017 and from: 08/03/2017 to: 13/03/2017	**Date of report**: September, 2017
Shifts: 18	**Local contact(s)**: Hiram CASTILLO-MICHEL (at ID21) and Vanessa Isabel TARDILLO SUAREZ (at ID16B)	*Received at ESRF:*

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Report:

Summary: Experiment LS-2638 has been an extension of LS-2499 to complete the data collection and confirm the preliminar results. In both experiments we used the combination of synchrotron radiation-based μXRF (ID16B-NA) and μFTIR (ID21) techniques to relate the severity of Alzheimer Disease (AD) dementia with characteristic secondary structures of Aβ amyloid peptide, elevated levels of metal ions (Cu, Zn, Fe, Ca) and lipid oxidation in human brain samples. On the one hand, the last results confirm the 'in situ' co-localization of AD plaques and lipid oxidation (as in previous studies[1,2]) found by μFTIR. Also, it was found an elevated presence of metal ions (Cu, Zn, Fe, Ca) on the plaque area which might be the cause of oxidation. This μXRF results are in agreement with other group results on human, transgenic mouse tissue[(3)], and rat[(4)]. On the other hand, we identified by Principal Component Analysis (PCA) of μFTIR data, two different types of amyloid aggregates in AD patients: Fibrillar aggregates with an intens band around 1627 cm^{-1} typical of β-sheet aggregates and unordered and beta aggregates with a less intens band at 1627 cm^{-1} and a clear band at 1647 cm^{-1}. Both were confirmed to be amyloid peptides by immunohistochemistry using an antibody anti-βamyloid peptide, but only dense plaques were detectable by ThS fluorescent probe of fibrilar structures. Identification of unordered amyloid aggregates absorbing at 1647 cm^{-1} in human brain tissue samples would be firstly described for human AD and could be a valuable tool for the identification of non-fibrillar structures directly on tissue samples, that might be early aggregate structures. We found dense and diffuse plaques in the same tissue but in different proportion depending on the patient.

Methodology: cryosectioned and cryo-dried human brain tissue samples were cut in contiguos slides of 8 μm thick of the cortex region afected by AD. Age-related brain tissue of nonaffected by AD of corresponding cortices were taken as a control. The senile plaques were localized by use of the fluorescent probe Thioflavine S (Th S), which becomes fluorescent when combining with aggregates. Then contiguos slices of the same region were mounted on Si_3N_4 windows suitable for μXRF and μFTIR analyses. The μFTIR map of dense and diffuse plaques and the tissue surrounding the plaques was measured with a 10×10 μm^2 μFTIR beam, step size 5 μm and 128 scans at each pixel. Spectra was acquired over the 700-4000 cm^{-1} region using the MCT-B detector. For μXRF imaging at ID16 the energy was fixed at 17.5 keV, so we could analyze Fe, Cu, Zn, Ca distribution in the same samples at 0.2 μm^2 pixel size. The strategy followed was first to map the amyloid deposits of AD tissue cortice slides by FTIR imaging at ID21, and then, the same regions were analyzed for metals by μXRF at ID16B beam line. A python script to transfer coordinates through references from the optical images allowed us to easily localize the same areas. Data was analyzed using OMNIC, PyMCA programs, and PCA was used for the statistical analysis (Unscramble-CAMO). Finally, they were analyzed histologically by ThS probe staining or immunohistochemistry using antibodies against amyloid peptides on the synchrotron measured samples and on the contigous slides of the same tissues.

Results: on the one hand the FTIR imaging data shows oxidized lipid in the plaques and in the tissue immediately surrounding the aggregates as it was previously shown [(2)]. On the other hand, it has been found a clear distinction of two different types of amyloid aggregates with a maximum at 1627 cm^{-1} and at 1647 cm^{-1} by analysis using Principal Component Analysis (PCA) (figure 1a, 1b, 1c). This is being shown for the first time in an 'in situ' human tissue analysis for AD amyloid deposits. The distinction between amyloid aggregates is a central point in relation to amyloid toxicity since toxicity is related to the formation of aggregates (oligomers, amorphous aggregates) structurally distinct from amyloid fibrils. Such a distinction has been largely explored in 'in vitro' studies of the amyloid oligomerization process, but is the first time found 'in situ' in human tissue. The histological identification by immunohistochemistry confirmed that both aggregates are amyloid when analysed with specific antibody anti-βamyloid peptide (figure 1d), and they showed the typical shape of dense plaques or diffuse plaques (figure 1d) in the case of aggregates absorbing at 1627 or 1648 cm^{-1} respectively. Nevertheless, when analyzed by Thioflavin S (ThS) only dense plaques were positive, indicative of fibrilar structures. We could find dense and diffuse plaques in the same tissue but in different proportion depending on the patient. Identification of unordered amyloid aggregates absorbing at 1647 cm^{-1} in human brain tissue samples would be the first described for human AD and could be a valuable tool for the identification of non-fibrillar structures directly on tissue samples, that might be early aggregate structures.

The µXRF results show that Fe, Cu, Zn, Ca and S ion maps co-localize with the plaques and their surroundings with a cation content in these regions well above the level measured in the controls (fig1e). The elevated sulfur (S) on plaques, could be explained by the Met-35 of amyloid peptides that are accumulated on plaques. Metal ion accumulation on plaques is in agreement with other groups results on human and transgenic mouse tissue[(3,4)].

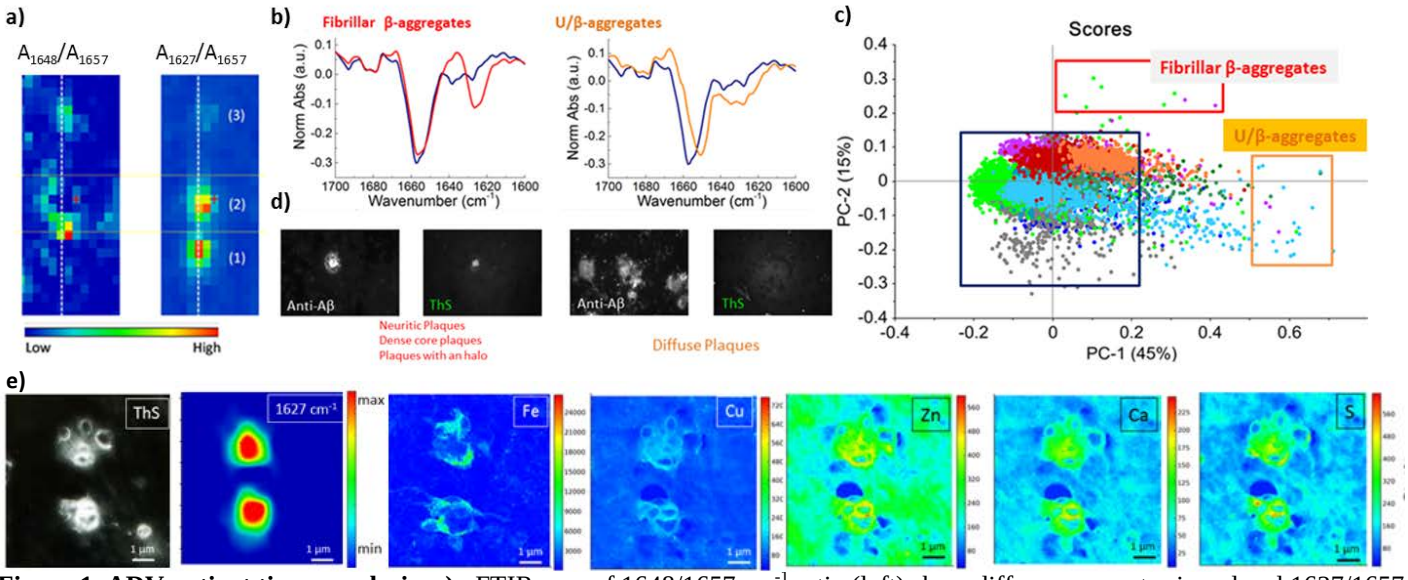

Figure 1. ADV patient tissue analysis. a) µFTIR map of 1648/1657 cm^{-1} ratio (left) show diffuse aggregates in red and 1627/1657 cm^{-1} ratio (right) show dense core plaques in red. **b)** PCA loadings (PC1 in blue and PC2 in red for fibrillar β-aggregates and orange for unordered β-aggregates). **c)** PCA analysis of the Amida I of controls, ADV, and ADVI. Outliers squared in red correspond to fibrillar β-aggregates, squared in orange correspond to unordered β-aggregates. **d)** anti-Aβ immunohistochemistry and ThS fluorescent probe test on neuritic plaques/dense core plaques/plaques with halo (on the left) and on diffuse plaques (on the right). **e)** plaques (2) and (3) marked on figure 1a are shown here, stained with ThS, µFTIR map of 1627 cm^{-1}, and µXRF showing Fe, Cu, Zn, Ca and S. For all maps intensities were expressed as red – maximum (25000 Fe cps, 800 Cu cps, 600 Zn cps, 250 Ca cps, 600 S cps) and blue – minimum (0 cps). A higher content in metal ion associated to amyloid plaques is detected for samples containing a higher content in 'low frequency amyloid aggregates' (peptide aggregates absorbing at 1627 cm^{-1} in the infrared).

From our study, the combination of the two techniques (µFTIR and µXRF), has made possible stablishing a new link between amyloid deposits, tissue oxidation and elevated metal cation levels 'in situ' for the first time. Because the relationship between structurally distinguishable amyloid aggregates, tissue oxidation and metal ion levels is of high significance in AD research, the results from experiments in ESRF' lines ID21 and ID16B are of great scientific value.

References

[1] Ferrer I. Prog Neurobiol 2012 Apr 97(1):38-51

[2] Benseny-Cases N. et al. Anal Chem 2014, Dec 16;86(24):12047-5

[3] Miller LM et al. J Struct Biol 2006 Jul;155(1):30-37

[4] Summers KL et al. Biochem 2017 Jun;56: 4107-4116