Multimodal synchrotron-based spectroscopy study of protein aggregation related to Alzheimer's disease

INDUSTRIAL PILOT PROJECT

THE INDUSTRIAL CHALLENGE

ReceptorPharma (RP) has previously generated promising preclinical data for a new drug candidate to combat Alzheimer's disease. However, there is still a need to assess and characterize drug-mediated changes of amyloid peptide aggregation – a central key player and presumed driver of the pathology. One goal is to visualize the subcellular localization of amyloid beta peptide aggregates and specific metal ions in a complementary manner, as well as to generate novel valuable data regarding RSs drug candidate.

WHY USING A LARGE SCALE FACILITY

To address protein aggregation directly in cells by spectroscopic approaches, we needed to employ synchrotron radiation sources that offer high flux brilliance necessary for the measurements to be performed at the submicron range (500 nm) with an acceptable signal-to-noise ratio. To resolve structural features of amyloid proteins in single cells, two complementary synchrotron-based techniques were used in tandem.

HOW THE WORK WAS DONE

With the ultimate aim study drug-mediated changes of amyloid peptide aggregation, cells were grown on either SI_3N_4 or CaF_2 sample supports treated with drug candidate or control compounds, after which the cells were fixed and stored until readouts could be performed. To investigate the mechanisms of amyloid aggregation (the role of trace metals related to Alzheimer's disease progression), X-Ray Fluorescence (XRF) was performed at the NanoMAX beamline of Max IV, Sweden. In order to address protein structures, Fourier Transform Infrared Microspectroscopy (FTIR) was performed at the SMIS beamline of SOLEIL, France. Also a novel technique, super-resolution infrared spectroscopy (O-PTIR), was employed at SIMS.

THE RESULTS AND EXPECTED IMPACT

First, we tested if FTIR/O-PTIR could be used to image amyloid structures directly in neurons (Figure 1). The FTIR/O-PTIR imaging results revealed polymorphic structures of amyloid aggregates at subcellular level. Based on our results, we suggest that polymorphic amyloid aggregates may trigger different mechanisms of AD progression. Second, we demonstrated that trace metals can be captured by XRF directly in neuronal cells (Figure 2). Third, we provided the first evidence that label-free super-resolution XRF and FTIR/O-PTIR can be used to image trace metals ions and aggregated amyloid-beta structures directly in the neuron in a correlative and complementary manner.

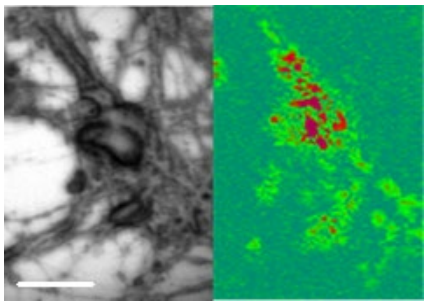

Figure 1. Example of a bright-field FTIR image of neurons grown on a CaF_2 window and the infrared map of proteins distributed inside the neuron. Scale is 20 µm.

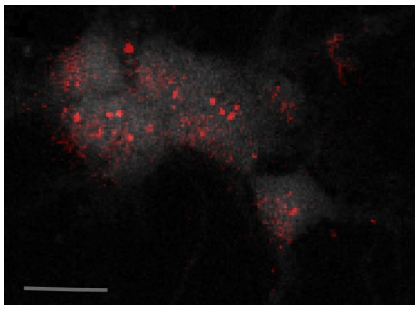

Figure 2. Example of XRF image Iron distribution in cultured primary neurons grown on a SI_3N_4 membrane for synchrotron XRF microscopy. Scale is 20 µm.

The main benefit for ReceptorPharma was that our staff has a possibility to learn synchrotron-based imaging techniques which could be used to assess and characterize drug-mediated changes of amyloid peptide aggregation at the level of a single cell. Moreover, a new approach was successfully tested. We believe that synchrotron O-PTIR/XRF may have a high impact for protein research community since it can likely be used also for other diseases where intracellular protein aggregation is aimed to be addressed.

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Vinnova's project No: 2019-02554 **Duration:** July 2019 -- June 2020

Funded by Sweden's Innovation Agency, Vinnova, in order to build competence and capacity regarding industrial utilisation of large-scale research infrastructures such as MAX IV and ESS.