DESCRIPTION FOR THE GENERAL PUBLIC

There were an estimated 50 million people worldwide living with dementia. The world's aging population is living longer and therefore the number of cases of debilitating and deadly neurodegenerative diseases, such as Alzheimer's still increases. The cost of treatment exceeded already 1% of the global GDP. Despite plethora of scientific efforts, there is no cure in sight. The neurodegenerative processes at the heart of Alzheimer's disease are caused by abnormal aggregation of cytotoxic, structurally abnormal proteins called amyloids. Therefore, the phenomenon of abnormal protein aggregation, responsible for the etiology of neurodegenerative diseases, is of central importance across a wide range of research disciplines. A better understanding of aggregation schemes of peptides related to particular diseases is extremely important because it can determine strategies for the early treatment. Based on the results of standard analytical techniques such as mass spectrometry, crystallography, circular dichroism, Fourier transform infrared spectroscopy and nuclear magnetic resonance it was possible to monitor conformational transition - an increase of β-sheet secondary structure content in aggregating peptides. However all of the methods listed above allowed modelling of only hypothetic amyloid aggregation pathways, but direct verification was prohibited due to methodological limitations. Each of the techniques requires significant volumes of the samples, and always delivers averaged (from many molecules) information about the secondary structure. Due to this high heterogeneity of the amyloid samples containing globular oligomers, immature protofibrils, mature fibrils, small aggregates such as dimers and tetramers, as well as non-aggregated peptides, until now it was not possible to follow every single step of the amyloid aggregation and to describe related changes in the amyloid secondary structure.

Here I propose a deep investigation into aggregation pathways of amyloid-β peptides related to Alzheimer's disease. This will be achieved by mapping of the β-sheet spatial distribution in individual nano-meter size protein aggregates. This approach, applied for the first time under conditions mimicking the physiological environment, will allow for direct verification of the peptide aggregation pathways. After a thorough analysis of the methods, which can probe molecular structure of individual nanometric aggregated I selected Tip-enhanced Raman spectroscopy (TER) technique since it meets all requirements of the proposed research, such as the ability to classify secondary structure, high sensitivity (down to single molecules), nano-metric spatial resolution, and the ability to perform measurements in liquids. TERS is a combination of Atomic Force Microscopy, which will allow for nanoscale proteins imaging and Raman spectroscopy for their chemical analysis. With TERS one can study chemical structure and composition with nano-metric spatial resolution. Additionally, I plan to monitor in the real time an influence of anti-aggregation agents on amyloids conformation by an introduction of Bexarotene into the buffer in which aggregating amyloid-β will be immersed. Fourier Transform Infrared nanospectroscopy (nanoFTIR) which is a complementary technique to TERS, will also be applied for comparison. An important part of the project is devoted to a deep investigation into the effect of amyloids on living neurons. I propose to apply Raman and infrared spectroscopies to follow molecular changes induced in mouse neuroblastoma by various amyloid-β forms (oligomers fibrils and protofibrills). A correlation between cytotoxicity of amyloids and their secondary structure will be deeply studied.

The project requires significant optimization of experimental methods, in particular the TERS technique for measurements in liquids. Optimized methodology could possibly be applied by chemists, biologists and biophysicists for investigation of many delicate biological systems such as chemotherapeutic drugs binding to DNA, the formation of domains in thin lipid layers under the introduction of membrane proteins and many others.

Above all, this project will gain essential knowledge about the abnormal aggregation of cytotoxic amyloids and molecular changes induced in amyloids upon treatment with anti-aggregation agents. Understanding the effects of intrinsic and extrinsic factors on the aggregation process at the molecular level is absolutely crucial for developing effective therapeutic strategies for Alzheimer's disease and inhibition the Alzheimer's peptides self-assembly process.