Abstract PhD thesis by Nils Skovgaard

It is currently known that a large number of proteins are involved in pathological protein aggregation in the brain, which is a common feature of many neurodegenerative diseases, including Parkinson's and Alzheimer's disease. Unique for these diseases are the large plaque-like accumulation of filamentous protein aggregates that eventually leads to a widespread loss of neurons, followed by malfunction and death of the patient. The filamentous protein aggregates, also known as amyloid fibrils, have a characteristic elongated, rod-like shape, rich on β-sheet structures. The fibril structure is the end result of a series of kinetic events. The process begins with misfolding of the amyloid proteins, which in turn release a cascade of structural re-arrangements and protein assembly mechanisms that eventually leads to the formation of fibrils. It is suggested that the overall fibrillation process is regulated via the establishment of equilibrium between multiple protein species, which co-exist in solution. Accordingly, it is not feasible to isolate individual species for structural characterization without interfering with this complex equilibrium, which ultimately might alter the structural properties of the individual species involved. Only little is known about the exact conditions that trigger the on-set of amyloid aggregations, the key factors that regulate the aggregation process, or the individual intermediate states of the amyloid proteins that is formed during fibrillation. Therefore, a better and more detailed knowledge on the fibrillation pathways and the exact role of individual protein species is needed in order to be able to pinpoint the toxic mechanisms that lead to neuronal death. Furthermore, a structural characterization of the individual protein species involved in the fibrillation pathways is crucial and a prerequisite for the establishment of new therapeutic strategies in the development of drugs that can delay or block neurodegenerative development. The overall aim of this thesis is to provide new ways in which we can reinforce the structural analysis of protein aggregates involved in amyloid formation. In particular, the fibril structure of alpha-synuclein (αSN), which is believed to play 3 a crucial role in cytotoxicity and development of Parkinson's disease, is brought into focus.

Microfluidic technologies represent a novel approach in which biochemical reactions can be performed and analyzed with use of very low sample volumes. Microfluidic-based technologies can be viewed as a toolbox by its own, which makes it possible to optimize sample preparation as well as sample handling and analysis in a unique system of micrometer-sized channels. In this thesis, experimental and technical approaches to develop microfluidic devices for the biological analysis of amyloid fibrils and mitochondrial proteins are presented and discussed. Our studies demonstrate how simple microfluidic devices can be established, which on the basis of small amounts of materials are able to both increase the investigator's control over the sample environment and to conduct a direct in-channel analysis of proteins. Regarding a structural characterization of amyloid fibrils, atomic force microscopy (AFM) is used to monitor changes in the αSN fibril stability during fibrillation. Among other things we wanted to assess the application of a morphological-based method in the investigation of the structural characterization of amyloid fibrils. Furthermore, atomic force microscopy and nano-resolution infrared spectroscopy (AFM-IR) are used to study the αSN fibril structure. These results are the first to provide an extensive conformational insight into the architecture of individual fibrils, and they demonstrate how advanced combinatorial techniques can benefit amyloid

research by allowing for a direct structural characterization of the protein species involved in the equilibrium-regulated aggregation process. The findings of the AFM-IR study show that it is possible to monitor changes in the fibril structure, and to relate these changes to the functional properties of individual species without relying on extensive decomposition of a signal average from a full ensemble of species. Data from AFM and AFM-IR based analyses show that the fibril structure undergoes large variations in the relative content of secondary structures during the late phase of the fibrillation process, despite maintaining a morphological resemblance. The large variations occurring in the fibril during the fibrillation 4 process causing changes structure stability suggest that the degree of completeness of the amyloid fibril structure may play an important role in relation to toxicity.

More than 10 million people suffer from Parkinson's disease worldwide, which underline the need for an efficient therapy. The challenges associated with investigation of the fibrillation pathways are abundant, which tempers the hope for an effective treatment within the near future. The thesis presented here examines some of these challenges and introduce new analytical approaches by which we might be able to obtain better and more detailed information on the structural properties of the individual protein aggregation species active in the brain. However, further studies are needed before a full characterization of the amyloid fibril structure and its role in neurodegenerative diseases is finally achieved.