Abstract

Despite the remarkable progress in modern medicine, numerous pathological conditions associated with aging (e.g. Alzheimer's, and Parkinson's diseases), due to their complex nature, remain uncurable. It is well-established that the aforementioned neurodegenerative diseases are linked with self-assembly (aggregation) of misfolded proteins – amyloids - which were found in the form of plaques or intracellular inclusions in *post-mortem* examined tissues. However, amyloids are structurally intricate and their role in pathology persists elusive. Therefore, the elucidation of the structural properties of amyloid fibrils is of great importance. The development of new amyloids bio-imaging techniques may provide insight into their formation mechanism, bio-functions (including toxicity), and, in further perspective, contribute to the progress in the development of novel therapeutic strategies.

This doctoral thesis presents the results of interdisciplinary research on amyloid bio-imaging, based on the selected imaging techniques (*i.e.* fluorescence, two-photon, polarized light, atomic force and electron microscopies) and exploring various aspects of imaging (*i.e.* multimodality, label-free imaging and functional labelling). Within the framework of this thesis, polarization-sensitive two-photon microscopy was utilized to image the organization of amyloid fibrils by analysis of conical distribution of two-photon excited emission dipole moments of amyloids intrinsic fluorescence (autofluorescence). As shown on an ordered model, namely amyloid spherulites, two-photon excited emission of amyloids is highly polarized and distributed in a 29° cone around the long fibril's axis. Therefore, I correlated the two-photon excited intrinsic fluorescence of amyloids with the orientation of fibrils in the sample plane, which is a promising tool for label-free amyloid imaging. Additionally, the data derived from the fluorescence imaging were supported with transmission electron microscopy (TEM) imaging. In detail, the ultra-thin cross-sections of fixed amyloid spherulites were investigated under TEM and the resulting images allowed to discuss the ultrastructure of the spherulite, *i.e.* to localize and describe regions of distinctive fibrils organization and amyloid structures contents. Correlated TEM and fluorescence imaging confirmed that the as-discovered polarization-dependent two-photon excited amyloid autofluorescence can report on and differentiate between well-developed amyloids fibrils from the amorphous structures, intermediate states or distorted fibrils. In general, the heterogenous internal structure of spherulites was successfully resolved. This discovery is of great significance and may contribute to a better understanding of amyloidogenesis at various levels of amyloid plaque formation. Last but not least, the scientific endeavors related to the synthesis, characterization, and application of ultra-small gold nanoclusters stabilized with a supramolecular ligand (12-crown-4 ether) for functional and multimodal amyloid imaging are presented. Presented herein nanoclusters are characterized by a molecule-like optical behavior (discrete electronic structure), near infra-red (NIR) fluorescence, amphiphilicity, and high electron density. Therefore, they were applied for amyloid spherulites and fibrils staining and imaging under fluorescence and electron microscopy, respectively. I showed that 12-crown-4 ether-capped gold nanoclusters exhibit a high affinity to hydrophobic bio-surfaces. In contrast to hydrophilic markers, my nanoclusters immediately bonded to amyloid spherulites and their pattering on

individual amyloid fibrils corresponds to the spatial distribution of domains with hydrophobic amino acids. Therefore, I presented the potential of nanoclusters as multimodal markers in the structural characterization of bio-interfaces based on its amphiphilic character.