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Amyloids: correlating autofluorescence in one- and two-photon regimes with morphology and secondary structure

Summary of the Doctoral Thesis

Amyloids are peptide or protein aggregates, characterized by the presence of the secondary β-sheet structure, stabilized by a dense hydrogen-bond network. They occur mainly in the form of elongated fibrils, and their deposition is related to some pathological conditions, including Alzheimer's disease. An interesting fact about amyloids is their peculiar property – autofluorescence. Upon excitation at 340-380 nm, they demonstrate the autofluorescence in the visible range of the electromagnetic spectrum (440-530 nm). Despite several hypotheses were put forward, the exact mechanism still remains elusive. Additionally, their autofluorescence properties upon a multiphoton excitation have been scarcely investigated. The improved knowledge of these non-linear optical properties could enhance the potential for *in vivo* detection of amyloid deposits.

This Doctoral Thesis covers interdisciplinary research of amyloid structures on the border of chemistry and materials science. The research involves (i) solid-phase peptide synthesis of amyloidogenic sequences and elaboration of the optimal incubation conditions, (ii) characterization of linear and non-linear optical properties, morphology, secondary structure of samples of interest, and (iii) molecular dynamics (MD) simulations.

Within the framework of this Doctoral Dissertation, the autofluorescence properties of amyloid fibrils formed from transthyretin 105-115 fragments, TTR(105-115), were investigated. The research covers enantiopure *L*- and *D*-samples along with their racemic mixture. The TTR(105-115) sequences were functionalized at the N- and C-termini with α-amino and amide groups, respectively. The morphology analysis indicated significant differences between the enantiopure and racemic fibrils. Interestingly, the racemic mixture exhibited a blue-shifted autofluorescence signal compared to both enantiopure analogues. In addition, the respective fibrils possessed different arrangements of the secondary β-sheet structure. The combined data provided evidence that variations in morphology and the organization of β-sheets can affect the autofluorescence properties of amyloid fibrils.

To better understand the correlations between the morphology and the secondary structure, the research was extended to other terminal functionalities of TTR(105-115). Indeed, the enantiopure and quasi-racemic samples significantly differed in terms of their morphology. The respective fibrils had different organization of the secondary β-sheet structure. The MD simulations evidenced that a sufficient number of hydrogen bonds is

required for amyloid fibrils to be formed. These outcomes demonstrate that modifications of peptides' terminal groups dictate the morphology and secondary structure of the resulting peptide aggregates.

The main research concerns also one- (1P) and two-photon (2P) excited autofluorescence of amyloids derived from hen egg-white lysozyme (HEWL), and correlations between the optical properties, morphology, and the secondary structure. Incubation of HEWL samples at varying ionic strengths led to the formation of amyloid fibrils differing in their morphology and the content of the β-sheet structure. Although the 2P excitation involved nearly twice the lower energy of the corresponding 1P process, the 1P and 2P excited emission signals had different relaxation pathways. The red-shifted 2P autofluorescence signal (compared to the 1P signal) was further confirmed by different fluorescence lifetimes for the 1P and 2P processes. These outcomes evidence that autofluorescence can be excited in a 2P process, which opens the possibility of detecting amyloids *in vivo*.

Overall, the results demonstrate that the interpretation of the morphology and secondary structure of amyloids helps to better understand the autofluorescence phenomenon and that amyloids can be investigated from different points of view, which encourages further research in the field.