Multilevel chirality, reversibility and polymorphism of amyloid fibril superstructures

Amyloid fibrils are supramolecular structures formed from native proteins in the off-pathway protein folding. Currently, it is assumed that the formation of amyloid fibrils is a generic feature of proteins. Amyloid fibrils are related with pathology of numerous amyloidoses, being, among other, a neuropathological hallmark of Alzheimer's disease. To date, more than fifty proteins associated with amyloid diseases have been identified. From the structural point of view, they are very unique and highly organized supramolecular assemblies with different levels of organization, including secondary protein structure, protofilament arrangement, and the architecture of mature fibrils (mesoscopic structure). An interesting feature of amyloid fibrils is their polymorphism, i.e., the tendency of amyloid fibrils to form various structures which depend among others on the preparation conditions. Another property that complicates fibril structures is their supramolecular chirality associated with the handedness of protofilaments and fibrils.

Among many important contexts for studying amyloid fibrils, there are two that are exceptionally essential. One is the context of fibril-related pathologies that have increasing incidence in the human population due to its rapid aging. From the point of view of amyloidosis prevention, it is necessary to understand the structural features of reversible oligomers and fibril polymorphs and identify the stages at the aggregation pathways leading irreversibly toward either amyloid fibrils or unorganized aggregates. Additionally, as recent experiments have shown the coexistence of two or more different types of protein aggregates in various neurodegenerative diseases, it is crucial to understand also the mechanisms of formation of fibrils from heterologous proteins.

The second very important context of studying amyloid structures is their use as nanomaterials. The ‘plastic catastrophe’ forces us to look for new green and biodegradable materials. Amyloid fibrils are not only biodegradable but also biogenic, formed in a self-assembly process, and have high potential for functionalization. For controlled synthesis of fibril superstructures of designed architectures and long-lasting stability, it is necessary to understand in detail their chirality, structural polymorphism, reversibility and the potential of various polymorphs for post-formation structural changes.

Therefore, the aim of the project is to shed light on several fundamental issues regarding amyloid fibrils, including their handedness and structural polymorphism, the reversibility of the formation of various structures on the off-pathway protein folding (from oligomers to mature fibrils), the capacity for post-formation structural modifications and alterations of fibril structures due to the addition of small amounts of ‘seeds’ (mature fibrils) of polymorphs of different structure that would be formed under the given preparation conditions or ‘seeds’ of a different protein than that used for fibrilization.

The approach proposed in the project is distinct in this sense that fibrils of native proteins differing in the secondary structure (α-helix, β-sheet, mixed and random coil) will be analyzed in a systematic fashion to evaluate the effects of various stimuli for fibril formation and modifications (pH, temperature, protein concentration, agitation, ionic strength, etc.). In addition, modifications of fibril structures after their formation, in particular the reversibility of the fibrilization process and a systematic assessment of the impact of ‘seeding’ on fibril structures, will be studied.

A multiparametric approach will be adopted for analysis, based on application of different complementary techniques to study in detail fibril samples. In particular, chiroptical spectroscopies, which are unique tools to probe chirality, will be used to characterize different levels of chiral fibril structures. These techniques will be coupled with high-resolution microscopic methods, enabling structural analysis of fibrils in nanoscale and in some cases also with the atomic resolution.

The rich data generated and analyzed in project in the systematic fashion for numerous polymorphic fibrils formed from proteins differing by the structure will be analyzed to reach conclusions regarding:

- impact of the fibrilization conditions and ‘seeding’ on the structure/handedness of various levels of fibril organization; relation between different levels of fibril architectures,
- impact of various parameters on reversibility and post-formation modifications,
- heterogeneity of fibrils and influence of fibrilization conditions on handedness and polymorphism,
- structures/polymorphism of amyloid fibrils at early stages of their formation and relation between them and structures of mature fibrils.

The results obtained in the model systems *in vitro* may provide a better understanding and control of fibril formation, what is crucial in light of amyloidosis prevention and for the design of stable fibril-based nanomaterials with desired properties.