

Amyloid Nanoscale Chemical Analysis by AFM-IR: structural basis of proteins aggregation and toxicity

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A wide class of human diseases and neurodegenerative disorders is due to the failure of a specific peptide or protein to keep its native functional conformational state and to undergo a conformational change into a misfolded state, triggering the formation of fibrillar cross- β sheet amyloid aggregates. During the fibrillization, several coexisting species are formed, giving rise to a highly heterogeneous mixture. Despite its fundamental role in biological function and malfunction, the mechanism of protein self-assembly and the fundamental origins of the connection between aggregation, cellular toxicity and the biochemistry of neurodegeneration remains challenging to elucidate in molecular detail. In particular, the nature of the specific state of proteins that is most prone to cause cytotoxicity is not established. Unraveling amyloid single species biophysical properties still represents a formidable experimental challenge, mainly because of their nanoscale dimensions and heterogeneous nature.

Here, we demonstrate that Atomic Force Microscopy (AFM) based techniques are capable to measure the properties of heterogeneous populations and to investigate the biophysical properties of amyloid aggregates at the single molecule nanometer scale. In particular, we establish Infrared Nanospectroscopy, simultaneously exploiting AFM and Infrared Spectroscopy (AFM-IR), as a versatile tool to characterize at the nanoscale the conformational rearrangements of proteins during their aggregation. For the first time, we are able to reconstruct the aggregation process and to link nanomechanical and structural properties of individual amyloidogenic species at the nanoscale. Conventional methods could not have provided this information with the same clarity. This could be central to study the aggregation pathway of proteins and to design molecules that could interfere with amyloid aggregation delaying the onset of misfolding diseases.