X-RAY SPECTROMICROSCOPY CAPTURES CROSS-β FIBRIL FORMATION

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Protein misfolding into non-native β-sheets, consequent aggregation and amyloid fibril formation are characteristics of numerous degenerative diseases, including Alzheimer's disease, transmissible spongiform encephalopathies (prion diseases), type 2 diabetes and cardiovascular diseases. Few methods, however, can detect the secondary structure of proteins in cells or tissues, leading to poor understanding of these diseases. We show that x-ray absorption near-edge structure spectroscopy of poly-L-lysine, prion protein and β-amyloid can identify the formation of cross-β structure, the assembly found in all amyloid fibrils, by the appearance of a peak, which is only observed after fibril formation. Cross-β fibril formation orders side chains and arranges C-H covalent bonds parallel to each other making them spectroscopically distinct. We called the peak associated with side chain C-H bonds "the amyloid peak", since we also found it in fibrils of misfolded prion protein and β-amyloid, but not in these proteins' normal forms. The amyloid peak is sufficiently distinct to envision the localization of cross-β structures with x-ray spectromicroscopy in affected tissues. In all three systems, water-containing amyloid fibrils are more sensitive to radiation damage than the properly folded molecules. We show with kinetic and dose-response studies that a radiation-enhanced peak may also characterize water-containing amyloid fibrils. With the observation of the amyloid peak and the radiation peak in isolated proteins we paved the way for the identification of cross-β fibrils directly in tissue.

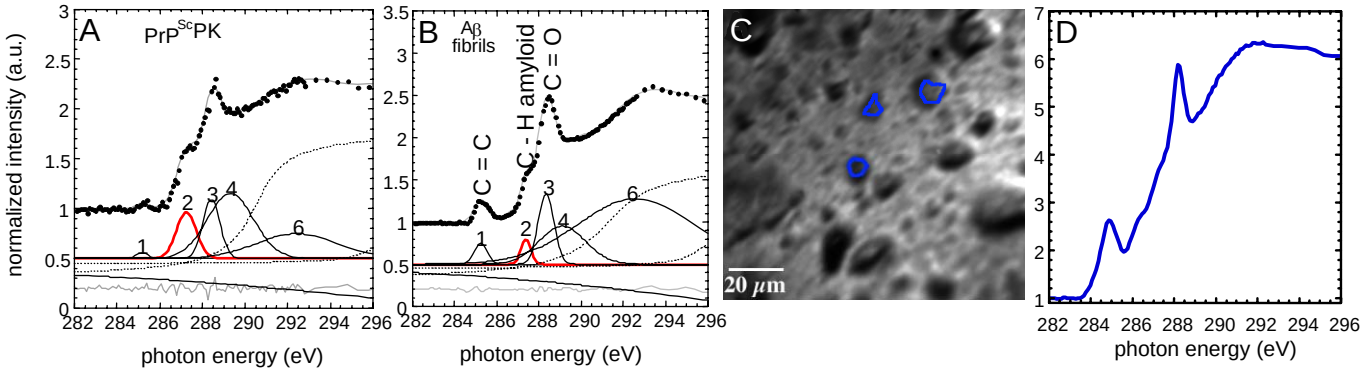

Figure 1. A-B: Carbon XANES spectra showing the amyloid peak (red) in prion protein and amyloid β. C-D: Future *in situ* identification of cross-β fibrils is possible. C: spectromicroscopic image of scrapie-infected hamster brain. D: Carbon spectrum extracted from the blue regions in C.