Nanoscale morphology and nanomechanical characterisation of recombinant human Amyloid-β 1-42 via tapping mode and ultrasonic force microscopies

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Alzheimer's disease and diabetes mellitus (type II) are part of a family of amyloid diseases caused by the miss folding and subsequent aggregation of the proteins amyloid-beta (Aβ) and amylin, and their accumulation in the brain or pancreas and subsequent cell death. The aggregation of the proteins follows a distinct hierarchy, with individual protein units joining together to form oligomers and continuing to grow through the protofibril stage until they reach mature fibrils. Little is known about the actual aggregation process or the nanostructure of such fibres, however information is available about the general morphology Full length fibrils are approximately 6-10 nm in diameter and consist of two or three protofibrils twisted together. As a result of the twisting the mature fibril frequently exhibits a helical twist, with a periodicity varying depending on incubation conditions. [1-4]. Aβ1-42 fibrils have been noted to be highly polymorphic, depending on the buffers used, pH, temperature at which the experiment is conducted, and also the SPM techniques utilised [5].

Given the soft nature of biological proteins amyloid fibres are most frequently imaged in tapping mode [6], as the oscillation of the tip reduces contact with the sample and also as the compressive force being applied to the sample in the tapping mode, is much easier for biological samples to withstand compared to shear forces in contact AFM. Nevertheless, it has been reported that when applicable (e.g. in liquids), contact mode produces highest resolution imaging of such fibres. The actual cantilever tip itself can also have a dramatic impact on the measurements taken, with the tip radius affecting the resolution of the image in the *xy* plane and distortion of the width of the fibres by indentation of the tip [2, 6].

In this paper work has also been conducted to elucidate a sample preparation method in volatile buffers which allows generation of physiologically relevant fibres while excluding the salt crystals which often contaminate otherwise ideal imaging conditions. We also present a new methodology of using Ultrasonic Force Microscopy (UFM) [7] for nanoscale imaging of Amyloid fibres at various stages of their aggregation. Although UFM is a contact AFM, with the tip-surface contact modulated at high frequency in MHz range that drastically reduces friction forces and shear stresses on the fibres. At the same time, UFM allows to map nanomechanical properties of the sample with high resolution that can be improved by using silicon cantilever tips of different levels of sharpness. In our measurements, tapping mode provided accurate structural information of the protein fibrils with current testing of super-sharp tips is underway. UFM provided new information about the elastic properties of the mature protein fibril during aggregation, including the helical structure of protofibrils arranged around a hollow core.

References

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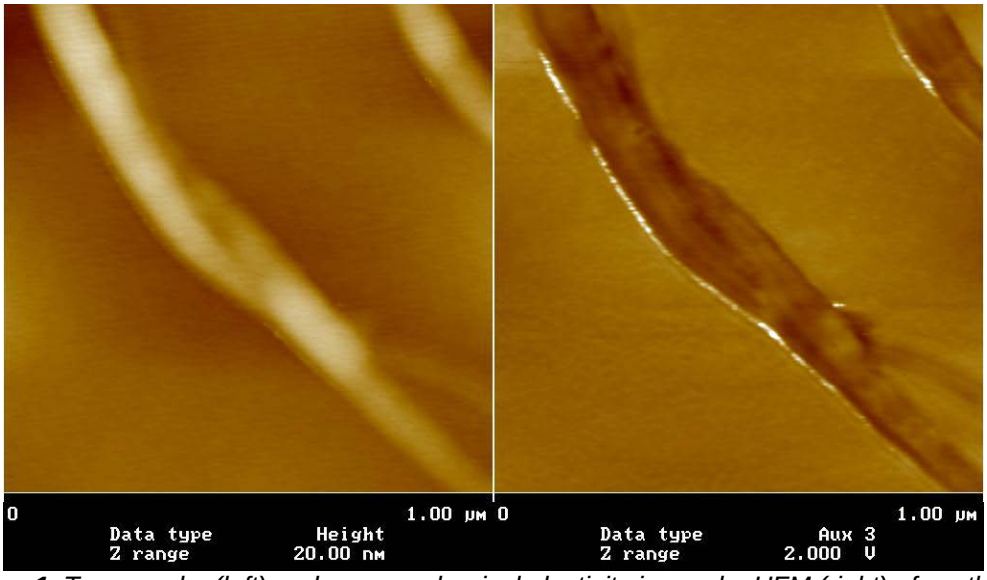

Figure 1. *Topography (left) and nanomechanical elasticity image by UFM (right) of synthetic human Amyloid fibre. Protofibril structure and domains are clearly seen in the elasticity image with resolution of the nanomechanical properties on the order of 15 nm.*

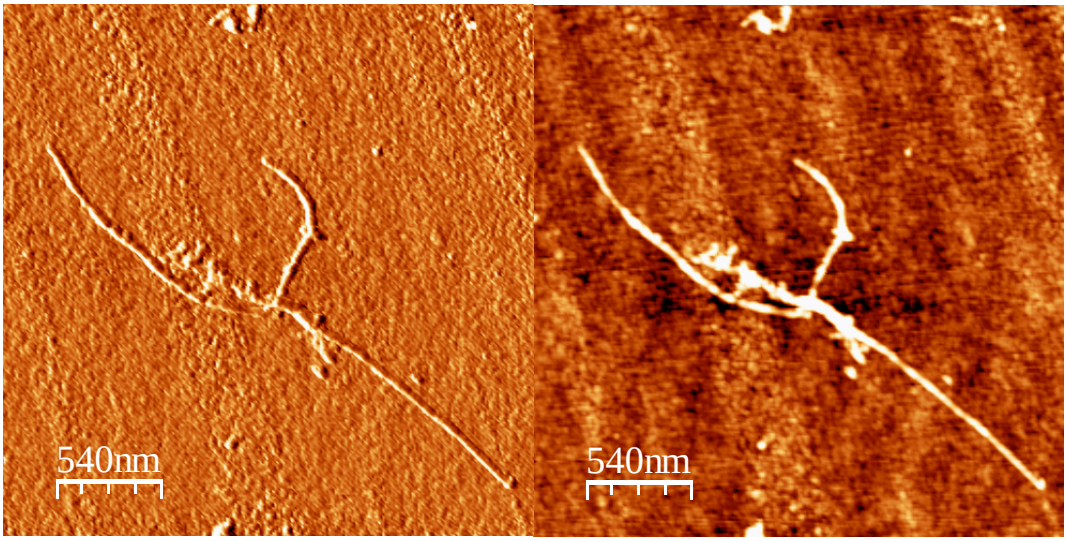

Figure 2. *Phase (left), and height (right) image of recombinant Aβ1-42 seen in tapping mode. Aggregation was monitored over 72 hours, and the growth of mature fibrils > 1µm long was seen. Protofibril structure and nanomechanical properties were also measured.*