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Near Field IR Microscopy of Amyloid Fibrils of β2-Microglobulin

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Amyloid fibril formation is caused by the aggregation of proteins and is associated with many neurodegenerative diseases, as well as debilitating ailments such as carpal tunnel syndrome. β2-microglobulin (β2-m) amyloid fibril formation is detected in patients with chronic renal conditions while undergoing hemodialysis.[1] β2-m amyloidosis occurs when the β2-m protein accumulates in high concentrations, thus forming amyloid fibrils that collect pathogenically in body joints. Amyloid fibrils, including those of β2-m, share three similar physical characteristics. First, these fibrils exhibit a high percentage of β-sheet structure in a "cross-β" pattern where the β-strands are perpendicular to the long axis of the fibril. Second, amyloid fibrils are exhibit unbranched structures micrometers in length. Last, these fibrils have the ability to allow a dye such as Congo red to intercalate between the β-sheets, yielding green gold birefringence that is observed under cross-polarized optical microscopy.

Near field IR imaging is a method to produce high resolution (<30nm) images with infrared light. We have developed a novel spectrometer for this measurement, seen in Figure 1. An atomic force microscope (AFM) is used for measuring the topography of the sample as well as for producing the near-field enhanced scattering modulated at the tip oscillation frequency. Tapping-mode, platinum coated cantilevers enhance the scattering of the continuous wave, tunable (frequency range, 2100 to 1600 cm^{-1}) IR laser near the surface. A helium neon laser guides the alignment of the invisible IR beam. The IR laser beam is directed toward a focusing lens after passing a partial reflector and focused onto the apex of the oscillating AFM tip. The polarization of the beam is parallel to the long axis of the probe. The backscattered IR radiation is then added to a reference homodyne field (reflected from a piezoelectric mounted mirror). A paraboloidal mirror focuses the infrared beam on a mercury cadmium telluride (MCT) infrared detector. The AC part of the detected signal is separated using a lock-in amplifier to demodulate the signal at the tip oscillation frequency from the static background. The scattering intensity is monitored as the AFM tip scans over the surface and the topography data is obtained simultaneously.

Figure 2 shows ANSIM images of β2-m fibrils collected at four different wavenumbers. Figure 2A represents a topography image collected simultaneously with a near-field image. The corresponding near-field images are provided in Figure 2B-E, where the numbers on the top left of the images indicate the specific IR wavenumber used. Figure 2B (1680 cm^{-1}) shows that fibrils of the same height scatter light to significantly different degrees, and therefore topography coupling is not dominant. Each label on the image in Figure 2A represents one fibril. The labels indicate TMAO-TFA fibril complex (T) and parallel β-sheet conformation (P). Attenuation at 1691 cm^{-1} is mainly due to TMAO-TFA fibril complex. Attenuation at 1631 cm^{-1} shows parallel β-sheet secondary structure. Attenuation at 1647 cm^{-1} shows random coil secondary structure. Attenuation at 1680 cm^{-1} shows primarily undefined, disordered secondary structure. Contrasting Figure 2D (1631 cm^{-1}) and Figure 2E (1691 cm-$^{1)}$, it is apparent that fibril T1 is mainly composed of TMAO-TFA fibril complex with parallel β-sheet secondary structure, whereas fibril P1 is composed of parallel β-sheet secondary structure. P1 shows much lower scattering at 1631 cm^{-1} but not at 1691 cm^{-1}, while T1 shows lower scattering at both wavenumbers. Similar

assignments are made for T2-T4 and P2-P5. Though the topography of the two fibrils is very similar (the height of both fibrils is approximately 10 nm) and the fibrils are spatially only a few nanometers apart, their distinct measured and predicted spectra indicate different compositions between different amyloid fibrils made from TMAO in solution.

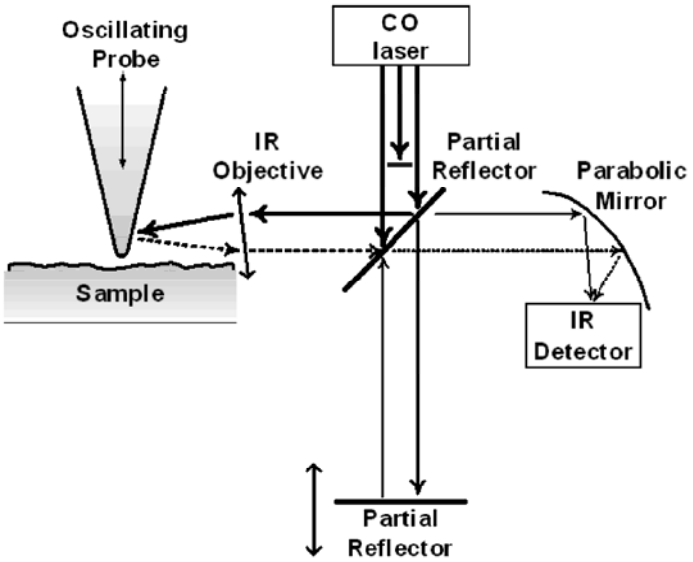

Figure 1. A diagram of the near-field IR microscope

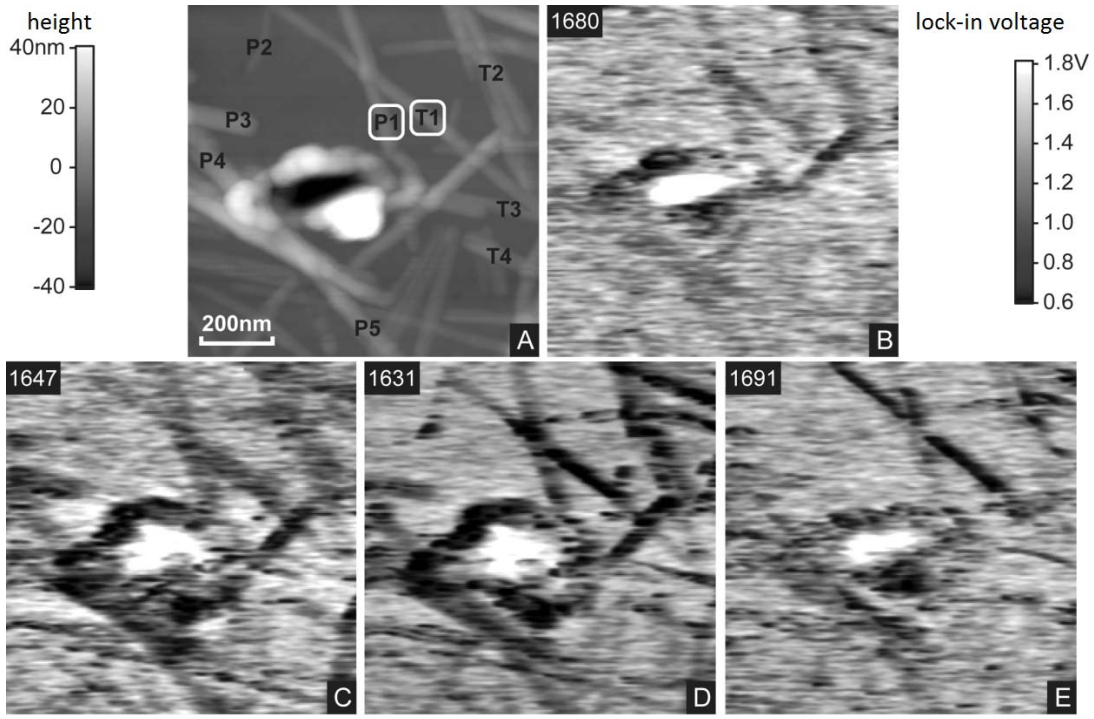

Figure 2. Height (A) and IR (B-E) images individual 21-31 peptide fibrils at indicated wavenumbers.

[1] G. V. Kozhukh, *et al* J. Biol. Chem. **277** (2002), p. 1310.
[2] M. Paulite, *et al* J. Am. Chem. Soc. **133** (2011), p. 7376.
[3] The authors acknowledge NSERC for financial support.

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