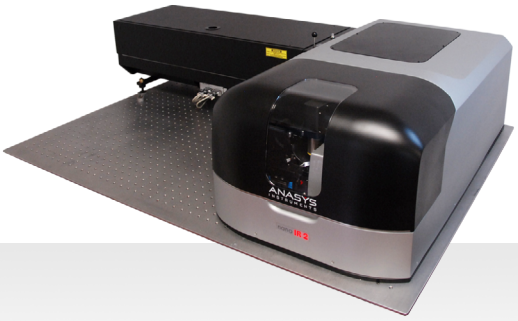
AFM-IR spectroscopy characterization of protein secondary structure in amyloid fibrils

Sub-diffraction limit spectroscopy for localized analysis of protein conformation



Introduction

Proteins, lipids and carbohydrates all have strong and diagnostic infrared (IR) spectra, making IR spectroscopy a standard tool in many life science applications ranging from fundamental biophysical research to applied food science. Infrared spectra are sensitive to local chemical environment, but conventional techniques result in an IR spectra that are an average of the entire sample due to insufficient spatial resolution. AFM-IR technology[1] makes it possible to obtain highly localized IR spectra correlated to sub-micron features within specimens promising new insights into the nature of biological structures and processes.

Protein Secondary Structure

The secondary structure of proteins (helix, sheet, coil, turns) is driven by hydrogen bonding among amide functional groups along the polypeptide chain. These intramolecular interactions influence the frequencies of amide vibrational modes, thus making IR spectra of the amide region a direct indicator of the state or change of secondary structure. Figure 1 shows spectra of model proteins illustrating how their IR spectra are influenced by secondary structure.

Unlike conventional FTIR, AFM-IR achieves spatial resolution necessary to observe secondary structure in localized regions of a sample. Figure 2 demonstrates high resolution nature of AFM-IR by imaging individual collagen fibril based IR absorbance of the Amide I band. The spectrum of collagen is an AFM-IR spectrum from a single point on the isolated fibril.

High resolution capability of AFM-IR can allows for a rapid analysis of biopolymer aggregates. Amyloid proteins form characteristic fibril structures with

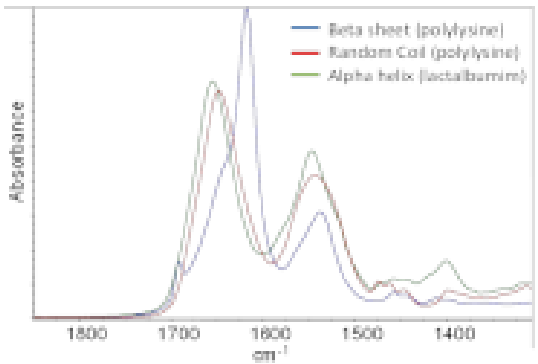


Figure 1. Bulk infrared spectra of proteins with differing secondary structure

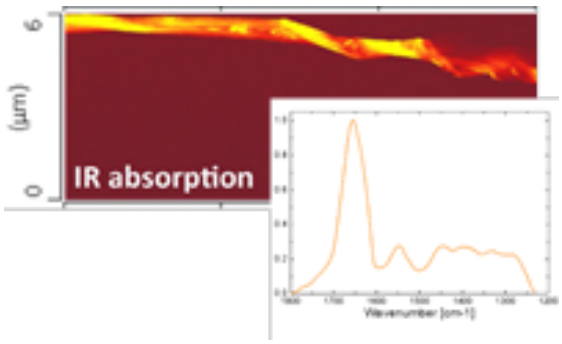


Figure 2. AFM-IR image and spectrum of individual, twisted collagen fibril. Unwound fibril thickness ~100 nm.[2]

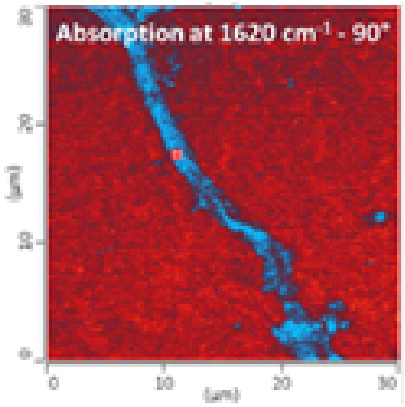


Figure 3. Polarized AFM-IR image of ERA17 amylodic peptide verifying consistent and oriented beta sheet conformation.[2]

beta-sheets oriented perpendicular to the main fibril axis. Figure 3 is AFM-IR image monitoring for secondary structure using both wavelength and polarization angle to enhance contrast for amyloid like beta sheet structures. The uniformity of the absorbance intensity indicates tightly packed and consistent amyloid growth of the fibril from the peptide monomers.

Study of secondary transformations between states of biopolymers is possible with AFM-IR. A recent study[3] analyzed aggregates of the Josephin domain of the ataxin-3, a protein domain implicated in a serious neuro-degenerative disorder. Figure 4A shows the infrared image of a growing fibril in presence of spherical oligomer aggregates. By monitoring the species over different incubation times, the oligomers show a clear increase in beta sheet secondary structure as they assembly into fibrils. Figure 4B compares the AFM-IR spectra of initial, intermediate and final forms during the process.

Reference

1. Kulick, et al., Microscopy and Analysis, May 2014

2. Ruggeri, et al., J Peptide Sci, DOI 10.1002/psc.2730 (2014)

3. Ruggeri, et al., Nature Comm., DOI: 10.1038/ncomms8831 (2015)

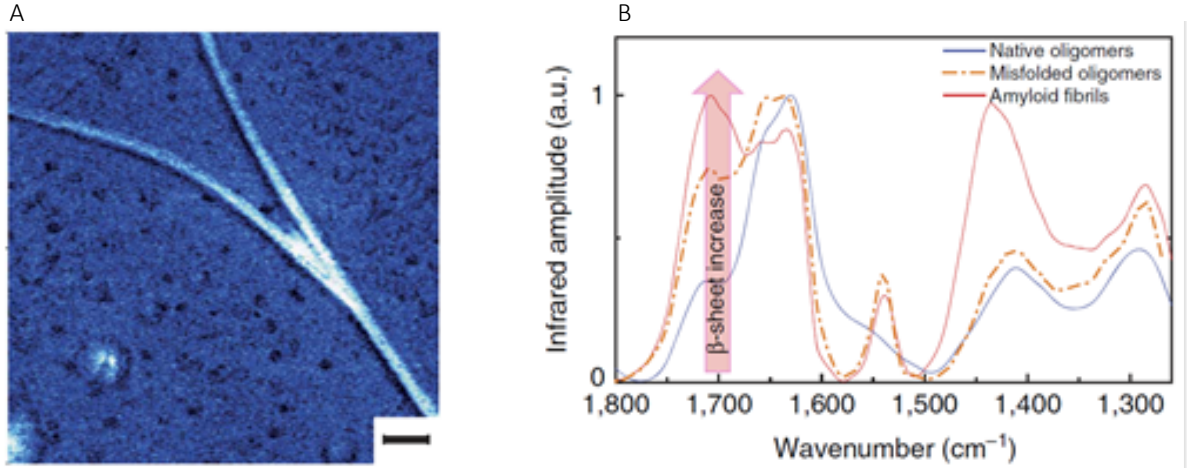


Figure 4. (A) Infrared absorbance image at 1655 cm-1 of growing amyloid fibril. Scale bar is 1 micron (B) Average AFM-IR spectra of protein phases at different points in fibril assembly.