Amyloid Fibrils: Electromechanical Imaging Using Bandwidth Excitation - Piezoresponse Force Microscopy

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Statement of Purpose: Amyloid fibrils are protein aggregates that have a definitive, non-centrosymmetric cross-β-sheet core structure. Most proteins can be induced to form amyloid fibrils, which have become associated with a family of diseases termed amyloidosis. A well-known example of localized amyloidosis is Alzheimer's Disease (AD). In AD, amyloid fibrils were suspected as the main culprit for approximately 15 years, yet recently oligomers of amyloidogenic proteins have been popularly advanced as the mediators of toxicity, perhaps regulating the amyloid fibers to an intended self-protection role in which the fibers are deposits of the more harmful oligomeric species, though this remains unresolved.

Two mechanistic pathways for amyloid toxicity in AD have been promoted. First is the suggestion that oligomeric species alter the electrical properties of the plasma membrane of a cell, leading to cell death. Second is evidence that oligomers and fibers bind to cell membrane receptors, possibly initiating a lethal intracellular cascade. For either way, elucidation of the intramolecular forces, formation mechanisms, structure, and mechanical properties of amyloid fibrils will reveal details about the interaction of amyloidogenic species with themselves, with membranes and with cell receptors, as well as aid in the application of amyloid fibers in technology. To investigate the internal electrostatic interactions within an amyloid fibril, we have performed Bandwidth Excitation-Piezoresponse Force Microscopy (BE-PFM) on insulin amyloid fibrils in air and in distilled water.

Methods: BE-PFM was performed using an Asylum Research (Santa Barbara, CA) MFP-3D Atomic Force Microscope (AFM) with an in-house developed MATLAB/LABVIEW data acquisition and control system. The MFP-3D tip holder allowed the tip to be directly biased in liquid. Measurements were performed using Au-coated SiN tips (Olympus TR800PB, 40 nm nominal tip radii).

For amyloid fibril samples, bovine insulin (Sigma-Aldrich #I5500) was reconstituted to 5 mg/ml in 10 mM HCl [1]. This solution was incubated at 80 ◦C for 48 h. The fibril suspension was diluted to 0.5 mg/ml protein and was purified by 15 centrifugations at 3000g for 1 minute each to remove smaller aggregates. 10 µl of a 0.05 mg/ml suspension was adsorbed onto freshly cleaved mica (EMS #71851-05) at room temperature for 1.5 minutes, followed by washing with copious amounts of Millipore water. Imaging was performed in air or in 1 mL Millipore water in a static fluid cell.

Results/Discussion: BE-PFM provides four outputs: piezoresponse amplitude, resonance frequency, piezoresponse phase and quality factor (Q). For the insulin amyloid fibril in Millipore-grade water (Fig. 1), the resonance frequency decreases on the fibril compared to the mica substrate, as would be expected for the softer biological material. However, the piezoresponse amplitude is higher upon the fibril than on the mica. Using the Q value and phase, the contribution of the mechanical properties of the fibril to the electromechanical signal is determined, thus allowing for resolution of the electrostatic interactions within the amyloid fibril.

Piezoresponse force microscopy (PFM) has been developed to the point that biological materials may be imaged in liquids and result in qualitative evaluation of biological material properties, especially by utilizing BE-PFM. The biological materials that have been imaged range from protein fibers to living cells. Further developments towards separating mechanical contributions from the electromechanical response signal would provide a means towards quantitative analyses. More robust and efficient electromechanical imaging could allow rapid pursuits into electromechanobiology.

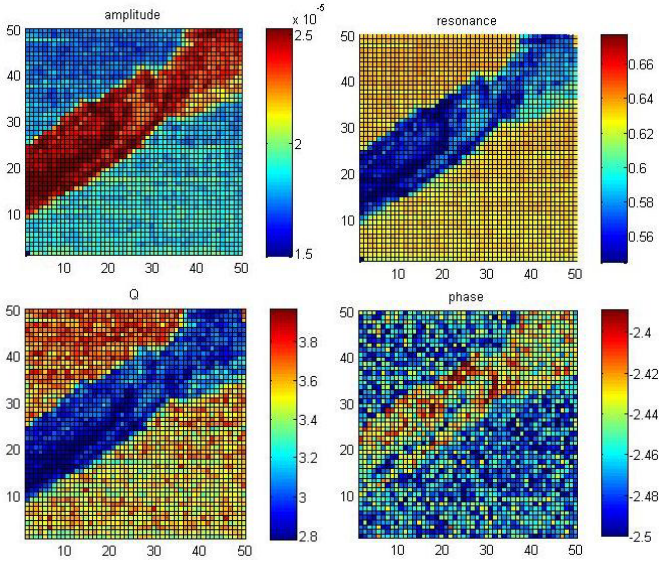

Figure 1. BE-PFM images of an insulin amyloid fibril. Scan size is 250 nm x 250 nm with 5 nm steps. Imaging was performed at 10 V_{app} with a 300 kHz bandwidth centered at 170 kHz.

Reference:
[1] Guo S. *Biomacromolecules*. 2006;7;1630.

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