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Identifying the Mechanism for Amyloid Formation Using Single-Molecule Spectroscopy TROY MESSINA, JASON GIURLEO, JONGJIN JUNG, HIYUN KIM, DAVID TALAGA, Rutgers, the State University of New Jersey — We are investigating the mechanism for the initial stages of protein self-assembly leading to amyloid growth using single molecule spectroscopy (SMS). β-lactoglobulin (β-LG) has been shown to form amyloid under denaturing conditions and has been chosen as a model protein for this study. Initial bulk experiments have been performed utilizing dynamic light scattering along with steady state and time-resolved fluorescence of conformationally sensitive fluorophores, and a preliminary mechanism of amyloid growth has been formulated. However, SMS directly identifies critical intermediates that may only be hypothesized by bulk experiments. A single molecule imaging experiment utilizing incubated samples of mono-labeled TMR-(β-LG) has been designed to count number of precursor monomers per aggregate species by counting the number of photobleaching steps required to extinguish a single particle's fluorescence. The time evolution of the particle number distribution is fit to a kinetic model representing a mechanism of amyloid growth. Results of bulk and single-molecule experiments will be discussed.

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