Multi-Dimensional Super-Resolution Imaging of Protein Aggregates Using Transient Binding of Single Fluorescent Molecules

PhD Dissertation Defense

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Abstract: Amyloid aggregates and tangles are signatures of various aging-related neurodegenerative diseases, such as Alzheimer’s disease and Parkinson’s disease. The aggregation of amyloid from small unit protein into amyloid fibrils is dynamic, transitory, and heterogeneous. Although many amyloid aggregates share a common structural motif, e.g., crossed-β sheets stacked together, not all aggregation structures are equally toxic. I present a method, transient amyloid binding (TAB) microscopy, for studying the heterogeneous organization of amyloid structures at the nanoscale. TAB microscopy resolves a variety of amyloid structures by using a standard amyloid dye, Thioflavin T, or a classic solvatochromic dye molecule, Nile red. Continuous transient binding of dye molecules to amyloid structures generates photon bursts for single-molecule localization over hours to days with minimal photobleaching, yielding ~40% more localizations than standard immunolabeling. With single-molecule sensitivity and long-term imaging capability, TAB monitors dynamics of the amyloid aggregates, such as elongation and remodeling, with nanometer resolution. I next extend TAB imaging to simultaneously measure positions and orientations of dye molecules bound to amyloid surfaces. I show that this new method, termed single-molecule localization orientation microscopy (SMOLM), robustly and sensitively measures the in (xy) plane orientations of fluorophores (~9° precision in azimuthal angle) near a refractive index interface when using a polarization-sensitive imaging system and joint sparse deconvolution algorithm. TAB SMOLM reveals the binding angles of Nile red molecules on amyloid fibrils, with a sampling density of ~2.3 molecule localizations per nm fibril length. The orientations of single fluorescent molecules also reveal structural heterogeneities along amyloid fibrillar networks that cannot be resolved by single-molecule localization alone.

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