Delineating the Mechanism of Heterotypic Multicomponent Phase Separation of Tau using Multicolor Fluorescence Imaging and Single-Molecule FRET

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Thesis Abstract

Biomolecular condensation has emerged as an effective means for cells to preserve their spatiotemporal coherence in carrying out a range of essential biological functions. These dynamics nonstoichiometric supramolecular assemblies are thought to form via phase separation of a multitude of intrinsically disordered proteins/regions (IDPs/IDRs) and other biomolecules into liquid-like membrane-less compartments. Aberrant liquid-to-solid transitions inside these phase-separated condensates are associated with a range of human diseases. The work described in this thesis dissects the crucial molecular events that govern the complex coacervation of tau with known cellular interactors to recapitulate the events that contribute towards modulating its phase behavior. The interactions of tau with unrelated amyloidogenic proteins hint towards the contributions of neuronal protein networks in the pathophysiology of overlapping neurodegenerative diseases. We showed that two neuronal proteins namely, tau and the prion protein (PrP), undergo complex coacervation fueled by domain-specific electrostatic interactions to form highly dynamic, mesoscopic droplets which, in the presence of RNA, can further be tuned to form multiphasic condensates reminiscent of hierarchically organized multi-layered intracellular bodies. To investigate the phase behavior comprehensively, we employed a unique combination of time-resolved methodologies in conjunction with multicolor fluorescence imaging and single-molecule FRET (Förster resonance energy transfer) that encompass a wide range of timescales. Using these tools, we dissected the crucial molecular events associated with the formation of heterotypic multicomponent condensates comprising transient, domain-specific, short-range electrostatic nanoclusters. We also showed that upon aging, tau:PrP heterotypic condensates gradually convert into solid-like hetero-assemblies via persistent intermolecular

interactions, which is the hallmark of overlapping neuropathological features. Using multicolor imaging and single-molecule FRET in conjunction with other biochemical and biophysical tools, we also delineated the effect of the protein quality control machinery on the phase behavior of tau. We dissected the molecular events associated with phase separation of tau in the presence of a chaperone (Hsp40) that abrogates the liquid-to-solid transition of tau into amyloid fibrils. In summary, the work described in this thesis contributes to our understanding of the role of biological phase separation in directing molecular networks orchestrated by IDPs and their modulation by molecular chaperones.