P-1.47

Insights into the Structure and Aggregation of C-terminal Domain of Tdp-43 and Its Als-associated Mutants

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TAR DNA-binding protein 43 (TDP-43) aggregates into intraneuronal cytoplasmic inclusions linked to amyotrophic lateral sclerosis (ALS), ubiquitin-positive frontotemporal lobar degeneration (FTLD-TDP), and limbic-predominant age-related TDP-43 encephalopathy (LATE-NC). The C-terminal domain (CTD) is crucial in the pathogenesis, as it is prone to aggregation and harbors most familial ALS mutations. This study aims to gain deeper insights into the structural properties, liquid-liquid phase separation (LLPS) and aggregation behavior of the CTD and its mutants. We purified wild-type (WT) CTD as well as a representative set of mutants associated with familial ALS. Brightfield and confocal microscopy showed that the WT CTD initially forms round droplets that give rise to fibrillar aggregates over time. Fluorescence recovery after photobleaching will assess the state of these droplets before solidification. The aggregation kinetics, monitored by turbidimetry and Thioflavin T fluorescence, revealed that WT CTD aggregates within 1 hour, potentially forming amyloid-like structures. By systematically comparing WT and mutant proteins under the same conditions, this study will provide insight into how mutations alter the structural ensemble, LLPS and aggregation properties of the CTD, with potential implications to elucidate disease pathogenesis and the involvement of the CTD.