Title:	Dissecting Molecular Events of Phase Separation of Fused in Sarcoma Using Single-Molecule FRET, HomoFRET, and Vibrational Raman Spectroscopy.
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Abstract:	Phase separation of biomolecules into liquid-like supramolecular assemblies has emerged as a critical organizing principle within living cells. Intrinsically disordered proteins/regions (IDPs/IDRs) comprising low-complexity (LC) and prion-like domains have been identified as the key candidates driving intracellular phase separation leading to the formation of noncanonical membrane-less organelles, also known as biomolecular condensates. These condensates are thought to spatiotemporal regulate critical cellular functions and are also known to undergo aberrant phase transitions associated with a range of neurodegenerative diseases. My thesis work illuminates the inner workings of an archetypical phase-separating protein, Fused in Sarcoma (FUS) using an amalgamation of single-droplet single-molecule FRET (Förster resonance energy transfer), picosecond time-resolved fluorescence anisotropy, fluorescence correlation spectroscopy in conjunction with vibrational Raman spectroscopy. For single-molecule FRET experiments, we chose the prion-like low- complexity domain of FUS (FUS-LC) which is the crucial driver of FUS condensate formation. Our results revealed the conformational distribution and dynamics within the monomeric and condensed phases at a single-molecule resolution in a droplet-by-droplet manner and demonstrated that the conformational unzipping is the key event that allows polypeptide chains to assemble via multivalent noncovalent interactions resulting in the formation of biomolecular condensates. A disease-associated mutation further facilitated chain expansion of the FUS-LC polypeptide chain, causing enhanced protein-protein interactions and an increase in dense phase concentration, leading to an increased aggregation propensity. We also demonstrated the power of vibration Raman spectroscopy to delineate the structural changes in the hydration water layer within protein liquid droplets. Our single-droplet vibrational Raman spectroscopy measurements highlighted a sequence-encoded reorganizationof the hydrogen bonding network of water molecules upon phase separation. Our study deciphered the internal restructuring of the solvent hydrogen bonding network distinctly within the condensates and the dispersed phase in a sequence-dependent manner. Finally, we demonstrated the application of homoFRET to study energy migration within the condensed phase of FUS both in vitro and in situ. Both steady-state and time-resolved fluorescence anisotropy measurements reported the extent of homoFRET that is used as a robust readout for the internal molecular packing within dynamic biomolecular condensates. This methodology allowed us to directly monitor the effect of modulators of phase behavior such as RNA and ATP, as well as the impact of post-translational modifications in protein phase separation. Using this methodology, we also used mammalian cell lines to study nuclear FUS and oxidative stress-induced stress granule formation in the cytoplasm. In summary, novel tools and concepts that are developed and utilized during my thesis work can have a wide range of applications in studying biological phase transitions involved in physiology and disease.
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