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Title:	Expression, Purification, and Characterization of Intrinsically Disordered Protein Regions Undergoing Phase Separation
Authors:	<a href="#">P G, Swastik</a>
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Abstract:	<p>Living cells are complex solutions of thousands of different proteins, nucleic acids, lipids and small molecules. To organize their contents, cells form many different types of intracellular organelles. In addition to canonical vesicle-like organelles, there are dozens of non- membrane bound, RNA and protein-rich organelles within the cell nucleus and the cytoplasm. Despite their lack of an enclosing membrane, these organelles are still able to concentrate molecular components and play important roles in key intracellular functions such as RNA transcription and processing, and the regulation of protein translation. Recent studies suggest that membrane-less intracellular compartments are condensed liquid like droplets of RNA and protein that form via phase separation. FUS, an RNA binding protein belonging to the FET family of proteins, is involved in many crucial functions of the cell including mRNA splicing and DNA damage repair. FUS consists of a low-complexity domain at its N-terminus which is rich in polar amino acids and depleted with hydrophobic amino acids. Mutations in certain domains of the protein have been shown to cause neurodegeneration and have implications in diseases like ALS and FTD. In this work, we have started to work on both the full-length and the low-complexity domain of the protein to understand the dynamics of the polypeptide chain inside the liquid droplet and the phase transition from liquid-like droplets to solid aggregates. We have also shown the role played by electrostatic forces and hydrophobic effects on the liquid-liquid phase separation of full- length FUS. To investigate the chain dynamics of the protein inside a liquid droplet we have successfully created six cysteine mutants at different locations of the sequence. Finally, we have worked hard to prepare all the ingredients required for our experiments and are now ready to make some intriguing observations using an array of biophysical techniques.</p>
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