Conformational properties integral to the phase separation properties of hnRNPA1 revealed by small angle X-ray scattering.

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Liquid-liquid phase separation (LLPS), a process in which a protein solution demixes into protein-dense and light phases, is likely critical in the formation of stress granules and other membrane-less organelles. Recent work further suggests that LLPS may promote protein fibrillization in neurodegenerative disease related processes, stressing the importance of a detailed understanding of the molecular interactions mediating LLPS and the conformational properties that predispose proteins to undergo LLPS. We are here using the RNA binding protein hnRNPA1 to tackle these questions. hnRNPA1 consists of two folded RNA recognition motif (RRM) domains and a long, intrinsically disordered domain with low sequence complexity, the so-called "low-complexity" domain (LCD). hnRNPA1 undergoes stimulus-responsive LLPS and forms condensed liquid droplets. While LLPS is intrinsically an intermolecular process, important contacts should be recapitulated intramolecularly in a monodisperse solution allowing us to probe the resulting structural features by small-angle solution X-ray scattering (SAXS). In this work, careful sample preparation and data collection removes the effects of intermolecular interaction. Ensemble modeling reveals two major conformations of hnRNPA1, in which the LCD either freely diffuses or is bound to the RRMs. The population of the compact conformation is enhanced at lower ionic strength, suggesting that the contact between the RRMs and the LCD is mediated electrostatically. The propensity for LLPS follows a similar trend, suggesting that the domain interactions contribute to function of full-length hnRNPA1. The LCD, which alone can mediate LLPS, samples compact conformations. This compaction seems to be driven by collapse of glycine rich regions and clustering of aromatic residues, as we reveal by combining SAXS with NMR spectroscopy. We expect that the molecular interactions we have revealed in hnRNPA1 are critical to LLPS, accentuating the power of SAXS to provide information on complex, dynamic protein interactions.