A Deep Dive into Biomolecular Condensates using Vibrational Raman and Single-Molecule Fluorescence

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Formation of biomolecular condensates via liquid-liquid phase separation of intrinsically disordered proteins/regions (IDPs/IDRs) and nucleic acids into membraneless organelles is involved in critical cellular functions. However, aberrant phase transitions are associated with debilitating human diseases. We discovered that the prion protein (PrP) (well-known for its association with mad cow disease and Creutzfeldt-Jakob disease) can undergo phase separation via weak, multivalent, transient intermolecular interactions between the N-terminal IDR that resembles a yeast prion-like domain. An intriguing disease-associated amber stop codon mutation (Y145Stop) of PrP yields a C-terminally truncated intrinsically disordered fragment. We demonstrated that Y145Stop spontaneously phase-separates into highly dynamic liquid droplets under physiological conditions [1]. Upon aging, these highly dynamic liquid droplets undergo a liquid-to-solid phase transition into highly ordered, beta-rich, amyloid-like aggregates that exhibit a characteristic autocatalytic self-templating behavior. The propensity for the aberrant phase transition is much lower for the full-length PrP indicating an evolutionarily conserved role of the folded C-terminal domain [1,2]. Our recent results also showed intriguing spatiotemporal modulations in complex coacervation of PrP with other neuronal IDPs (alpha-synuclein and tau) into heterotypic, multicomponent, multiphasic, multilayered condensates in the presence of RNA. These multicomponent condensates can act as reaction crucibles to catalyze the amyloid conversion of these functional assemblies into pathological aggregates associated with overlapping neuropathological features [3,4]. If time permits, I will also discuss our new method by which we can capture exquisite molecular details of biomolecular condensates using ultrasensitive surface-enhanced Raman scattering [5].

1. "An intrinsically disordered pathological prion variant Y145Stop converts into self-seeding amyloids via liquid-liquid phase separation". A. Agarwal, S.K. Rai, A. Avni & S. Mukhopadhyay. ***Proc. Natl. Acad. Sci. USA*** (2021) 118, 45, e2100968118. https://doi.org/10.1073/pnas.2100968118
2. "Prion Protein Biology Through the Lens of Liquid-Liquid Phase Separation". A. Agarwal & S. Mukhopadhyay. ***J. Mol. Biol.*** (2022) 434, 167368. https://doi.org/10.1016/j.jmb.2021.167368
3. "Spatiotemporal Modulations in Heterotypic Condensates of Prion and α-Synuclein Control Phase Transitions and Amyloid Conversion" A. Agarwal, L. Arora, S.K. Rai, A. Avni & S. Mukhopadhyay. ***Nature Communications*** (2022) 13, 1154. https://www.nature.com/articles/s41467-022-28797-5
4. "Heterotypic electrostatic interactions control complex phase separation of tau and prion into multiphasic condensates and co-aggregates" S. K. Rai, R. Khanna, A. Avni & S. Mukhopadhyay. ***Proc. Natl. Acad. Sci. USA*** (2023) 120, e2216338120. https://doi.org/10.1073/pnas.2216338120
5. "Single-Droplet Surface-Enhanced Raman Scattering Decodes the Molecular Determinants of Liquid-Liquid Phase Separation" A. Avni, A. Joshi, A. Walimbe, S. G. Pattanashetty & S. Mukhopadhyay. ***Nature Communications*** (2022) 13, 4378. https://www.nature.com/articles/s41467-022-32143-0