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学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　博士（情報科学）　氏名　田　雅

学 位 論 文 題 名

Raman Spectroscopy for Spatial Heterogeneity in Membrane-less Organelles

（ラマン分光法を用いた非膜オルガネラの空間的不均一性解析）

Membrane-less organelles (MLOs) are formed via liquid-liquid phase separation (LLPS) within cells. MLOs play a critical role in various cellular processes and exchange molecules quickly with surroundings. Moreover, the formation and dynamic behaviors of MLOs have significant implications for understanding human health. Disruptions in their formation and alteration in their dynamics have been linked to several diseases. Specifically, MLOs exhibit abnormal diffusion dynamics contributing to impaired functions with neurodegenerative diseases. Understanding the underlying molecular mechanisms of the diffusion dynamics and their contributions to disease development could be the key to unlocking novel therapeutic strategies targeting MLOs in neurodegenerative diseases.

Neurodegenerative diseases are characterized by the loss or dysfunction of neurons in the human brain. Two common examples are Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD), both of which have been linked to abnormal repeat expansions of *GGGGCC* sequences within the intron of mutant *C*9*ORF*72 gene. These hexanucleotide repeat expansions are translated via a non-ATG mechanism into toxic arginine-rich dipeptides, which could accumulate within various MLOs via LLPS and alter the diffusion behaviors. The diffusion dynamics and spatial heterogeneity within these LLPS-derived MLOs remain poorly understood.

In Chapter 2, we investigated *in vitro* liquid droplets formed by positively charged arginine-rich dipeptides, (PR)20 (twenty repeats of proline–arginine) mixed with negatively charged homopolymeric adenine (poly-A RNA), with spatially resolved fluorescence recovery after photobleaching (FRAP) analysis and Raman spectroscopy. The result was compared with an artificially designed dipeptide, (P4R4)5, which has the same number of proline and arginine but with the uneven charge pattern consisting of five repeats of four continuous proline and four continuous arginine. Although (PR)20 and (P4R4)5 have the same net charge, FRAP analysis revealed the reduced fluidity in (P4R4)5 / poly-A RNA compared to (PR)20 / poly-A RNA droplets, which is mainly caused by the uneven partition of the positively charged arginine in (P4R4)5 dipeptide leads to the higher binding energy compared to repetitive structure of (PR)20. Our finding revealed that Raman spectroscopy effectively enables the monitoring of the spatial gradient of RNA concentration inside individual droplets, which can be correlated with FRAP analysis. These positively charged arginine-rich dipeptides concentrate within liquid droplets and play a crucial role in forming hierarchical structure within liquid droplets. Taken together, Raman spectroscopy provides insights into understanding the diffusion dynamics and a more comprehensive understanding of their spatial distribution and molecular diffusion.

To further understand the mechanism regulating the diffusion behaviors of charged molecules in MLOs, in Chapter 3, we employed Raman spectroscopy and FRAP analysis to elucidate the spatial distribution and molecular diffusion behaviors of the dipeptides with different charge properties and poly-A RNA within LLPS-derived liquid droplets *in vitro*. Although (PR)20 and (GR)20 have the same partition of the repeat sequence and net charge, their sequence-specific charge distribution would influence the dynamics of LLPS-derived liquid droplets. Raman spectra of individual LLPS liquid droplet revealed that (GR)20 / poly-A RNA complexes have a higher density of RNAs than (PR)20 / poly-A RNA droplets. Furthermore, we observed a concentration gradient of RNA within individual liquid droplets in Raman spectra maps, while the intensity ratios between dipeptide and RNA remained uniform, suggesting a hierarchical structure and the formation of stable RNA-

dipeptide complexes. FRAP analysis of RNA and various small dye molecules with different net charges further indicated heterogeneous diffusion dynamics, with slower diffusion at the droplet center compared to the inner boundary. This trend aligns with the observed concentration gradient of RNA and dipeptides in the Raman spectroscopic measurements. Notably, only the negatively charged fluorescein showed a significant difference in the ratio of center-to-boundary recovery time between (PR)20 and (GR)20 droplets, suggesting stronger interaction with (GR)20.

Together, these findings reveal spatial heterogeneity and diffusion behaviors within LLPS-derived liquid droplets and highlight charge-dependent interactions in LLPS droplets. These results provide insights into the mechanisms governing molecular diffusion dynamics, contributing to a deeper understanding of protein aggregation in neurodegenerative diseases.