

VUV-CD Imaging of LLPS Proteins

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Proteins that undergo liquid–liquid phase separation (LLPS), such as (FUS), have been implicated in neurodegenerative diseases like amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) [1, 2]. However, the relationship between phase separation behavior and protein structure remains poorly understood. Clarifying how protein structural properties relate to their droplet state is essential for advancing knowledge of the molecular mechanisms underlying these diseases.

Our recent studies using vacuum-ultraviolet circular dichroism (VUV-CD) spectroscopy have demonstrated that the structure of FUS-LC changes in response to LLPS [3]. In the dispersed state, the protein is predominantly disordered, while its β -strand content increases upon phase separation. The emergence and development of amyloid-like β -structures into fibrillar assemblies are believed to contribute to the pathogenesis of neurodegenerative diseases. However, direct evidence linking these structural transitions to disease mechanisms remains lacking. A significant limitation has been the millimeter-scale footprint of the synchrotron radiation beam used for CD measurements, which has hindered quantitative evaluation of structural changes specifically within the droplets.

This study analyzed structural changes within FUS droplets using a synchrotron microbeam with a diameter smaller than the droplet size. The droplet state of FUS was reproduced, and scanning measurements were conducted using the newly installed microbeam VUV-CD imaging system at BL12. Protein samples were prepared to remain dispersed at room temperature and to form droplets below 5°C. The droplets were also observed by an optical microscope (Figure). The sample cell was scanned with a VUV beam focused to several tens of micrometers. CD spectra were successfully obtained from regions presumed to be within the droplets. Ongoing experimental trials aim to quantify changes in CD spectral intensity within the droplet phase.



500 μ m

FIGURE 1. The picture of LLPS of FUS-LC below 5°C.

REFERENCES

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