Label-free observation of liquid-liquid phase separation in vitro and in a living cell using Raman microscopy

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We performed Raman imaging of liquid droplets formed via liquid-liquid phase separation (LLPS) in a protein buffer solution, as well as stress granules formed in living cells subjected to oxidative stress. While LLPS plays essential roles in a variety of intracellular events, liquid droplets are also considered to be involved in protein aggregation in neurodegenerative diseases. To elucidate the nature of liquid droplets and reveal the relationship between LLPS and aggregation of proteins, quantification of a single droplet is essential. In this study, for the label-free quantification of LLPS, we obtained Raman spectra of inside and outside of a liquid droplet and estimated the protein concentration of the droplet using the water Raman band as an internal standard. We found that ataxin-3, which has a poly-glutamine chain on its C-terminal portion and is considered as the causative protein of Machado-Joseph disease, exhibits LLPS in buffer solutions containing crowding agents such as polyethylene glycol (PEG) and dextran (DEX)[1]. The Raman spectra of liquid droplets of ataxin-3 show that only water and protein existed inside the liquid droplets, while the outside was consisted with water and crowding agents. Based on the intensity of the Amide I band of protein (1660 cm^{-1}) inside a droplet and the O–H stretching band of water (3100-3700 cm^{-1}) outside droplets, we estimated the concentration of proteins inside a single droplet. The protein concentration varied depending on the concentration of crowding agents; the higher the concentration of PEG was, the higher the concentration of protein inside the droplets. We also performed Raman imaging of liquid droplets of the low-complexity domain of FUS, and found that the concentration of FUS inside a droplets varies depending on the surrounding

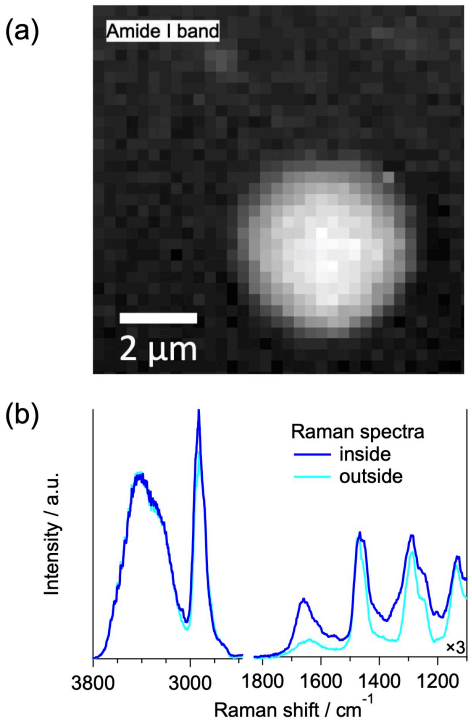

Fig. 1 A Raman image (a) and Raman spectra (b) of a liquid droplet of ataxin-3 formed in buffer solution containing PEG. The Raman image was obtained by mapping the intensity of Amide I band (1660 cm^{-1}).

circumstance, such as salt concentration and pH. These results indicate that the intracellular environments are important for LLPS inside a cell and the nature of liquid droplets varies with the intracellular molecular crowding environments.

As a demonstration of label-free observation of intracellular LLPS, we obtained Raman images of HeLa cells subjected to oxidative stress by addition of sodium arsenite into a medium, which induces stress granule formation. After 30 min. exposure to 0.5 mM sodium arsenite, the intensity of Raman bands assigned to proteins and nucleic acids was increased in some regions of cytoplasm. It is known that proteins and RNAs are the main components of stress granules, so that we concluded that we succeeded in obtaining Raman spectra of stress granules. The hierarchical cluster analysis (HCA) of Raman images consisting of 2500 Raman spectra enables us to visualize the distribution of stress granules. The HCA of Raman images also revealed that the intensity of the C–H stretching band increased not only inside the stress granule regions but also the other cytoplasmic regions. Since the intensity of the C–H Raman band corresponds the concentration of biomolecules having C–H bonds[2,3], the increase of the C–H Raman band indicates that the intracellular environments became more crowded in the entire cytoplasmic region after the oxidative stress. The change in the intracellular crowding environments will affect the formation process and change the nature of stress granules.

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