Protein Assembly Formed by Cooperative Optical Trapping at Solution Surface

National Yang Ming Chiao Tung Univ., Taiwan[1], NAIST[2], KU Leuven, Belgium[3], Kobe Univ.[4]

○Wei-Hsiang Chiu[1], Po-Wei. Yi[1,2], Roger Bresolí-Obach[3], Johan Hofkens[3], Eri Chatani[4], Shuichi Toyouchi[1], Hiroshi Masuhara[1]

E-mail: aa262435@gmail.com

Optical trapping is a powerful technique for manipulating nano- and microscale objects such as particles and molecules. We have been studying optical trapping of a protein, lysozyme, at the solution surface, where a highly concentrated lysozyme assembly formed and further extended to the outside of focus.[1] This phenomenon has been considered to be one kind of "Liquid-Liquid Phase Separation (LLPS)" which is a vital process that touches various cell functions.[2] In this work, we extended the trapping targets from single component protein solution to mixed solution by adding polyethylene glycol (PEG), which is a well-known crowding agent. The cooperative trapping behavior has been investigated by applying transmission/fluorescence imaging and Raman micro-spectroscopy.

A 1064 nm laser was tightly focused at the solution surface of 6.5 mM lysozyme D_2O solution. For such relatively low concentration of lysozyme, it led to no protein assembly formation even the trapping laser irradiation over 10 min (Fig. 1b). However, by individually adding 6.5 mM and 13 mM of PEG, a protein assembly formed at 5 min and 1.5 min of trapping laser irradiation and expanded with time at the surface of mixed solution (Fig. 1a, b). These results suggest that the large excluded volume of PEG as a crowding agent modifies lysozyme protein-protein interaction [3] which has increased the amounts of Lysozyme molecule clusters. Compared to molecules, these clusters have larger volume, which receives stronger gradient force. Namely lysozyme assembly is cooperatively facilitated under optical trapping. In the presentation, we will discuss the PEG crowding effect and cooperative protein assembling with Raman microspectroscopy results. This work will offer a new optical tool for investigation of protein LLPS and the molecular crowing effect with high spatiotemporal resolution.

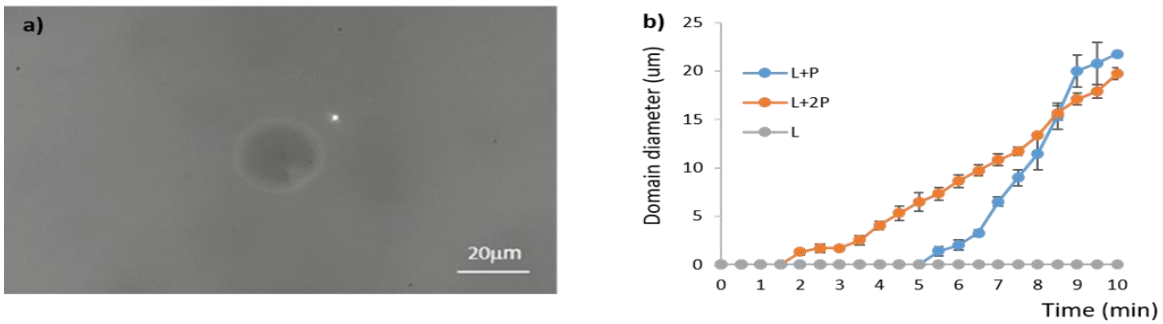

Figure 1. (a) A transmission image of a mixture of 6.5 mM lysozyme and 6.5 mM PEG at 10 min irradiation of trapping laser. (b) Temporal change in protein assembly diameter under optical trapping of L: 6.5 mM Lysozyme solution (gray), L+P: 6.5 mM lysozyme and 6.5 mM PEG mixture (blue), 2P: 13 mM PEG mixture (orange).

References. [1] P. W. Yi *et al.*, *J. Phys. Chem. C* **2021**, *125*, 18988. [2] A. A. Hyman *et al.*, *Annu. Rev. Cell Dev. Biol.* **2014**, *30,* 39. [3] K. Julius *et al.*, *Macromolecules* **2019**, *52*, 1772