## Cooperative Optical Trapping Dynamics of Protein and Polyethylene Glycol Studied by Fluorescence Imaging and Raman Scattering Micro-spectroscopy

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Optical trapping is known as a non-mechanical method of manipulating microparticles (MPs) and living cells in solution, while our group has demonstrated disk-like structure formation of the diameter of few ten micrometers of nanoparticles (NPs) and proteins<sup>1</sup> along the solution surface. Cooperative optical trapping of two materials (polystyrene NP, Au NP, polymer, and protein) does not simply give their mixture assembly but shows their characteristic organization. In the case of lysozyme and polyethylene glycol with 20,000 degrees of polymerization (PEG-20k), the latter macromolecular crowding agent accelerates the lysozyme assembling at and around the focus.<sup>2</sup> Here we report its spatio-temporal characteristics revealed by fluorescence imaging and Raman scattering micro-spectroscopy.

In Figure 1a, lysozyme assembly in the 375 mg/mL solution is visualized by scanning confocal fluorescence imaging of 0.03% added RhB-bonded lysozyme, which is widely expanded around the focus. The half of lysozyme is replaced by PEG-20k and the observed result is given in Figure 1b. The lysozyme distribution is narrower and shows sharp gradient, although the lysozyme concentration is half. The results are supported by Raman spectra and their non-negative matrix factorization analysis, which is now systematically extended. A new cooperative trapping behavior will be discussed.



**Figure 1.** (a) Fluorescence images of lysozyme solution containing dye-bonded lysozyme during laser trapping at the 375 mg/mL solution surface. (b) Fluorescence images of lysozyme (187.5 mg/mL) containing dye-bonded lysozyme and PEG-20K (187.5 mg/mL) mixed solution at its surface.

- 1. Po-Wei Yi et al., J. Phys, Chem. C, 125, 18988 (2021).
- 2. W.-H. Chiu et al., JSAP Spring Meeting (2022)