

Optically accelerated crystallization of supramolecular protein molecules under laser trapping

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Optically induced crystallization has been attracting much attention. Laser trapping is a potential approach to make the crystal of small molecules optically with spatiotemporal controllability. Tight focusing of a near-infrared (NIR) continuous wave (CW) and/or pulse laser to the air/solution interface of amino acid/D₂O solution can induce rapid crystallization from the focal spot^[1]. Crystallization of small biological molecules such as amino acid is possible, however, that of large biological molecules, i.e. proteins, by laser trapping is still difficult. Recently we have found that laser trapping with NIR CW laser at the air/solution interface of a hen egg-white lysozyme (HEWL)/D₂O solution ([HEWL] = 40 mg/mL, NaCl = 2.0 wt%, pD = 5.1) induces assembly formation, many micrometer-sized liquid droplet formation and, eventually, enhanced crystallization after liquid droplet formation/disappearance within a few hours.^[2]

We recently succeeded *in-situ* real-time crystallization of spherical supramolecular protein, ferritin (Fer8) under laser irradiation. Focusing of the trapping laser to the air/solution interface of the Fer8/D₂O solution ([Fer8] = 10 mg/mL, 0.5M (NH₄)₂SO₄, CdCl₂ = 0.2 wt%, pD = 7.0) induced the crystal generation from the focal spot within a few tens of minutes of trapping laser irradiation (Fig. 1a). Laser-induced crystals showed faster growth rate than that formed by spontaneous nucleation without laser irradiation (Fig. 1b). Fast crystal growth in the laser irradiated solution implies that laser-induced Fer8 concentration elevation since crystal growth speed increases depending on the protein concentration. Raman, polarized Raman and fluorescence microspectroscopy/imaging of crystal generation/growth process indicates the local concentration elevation of Fer8 at and around the focal spot under trapping laser irradiation. These results indicate that symmetric Fer8 can satisfy required molecular orientation and alignment for the crystallization in optically achieved local high concentration domain at the focal spot.

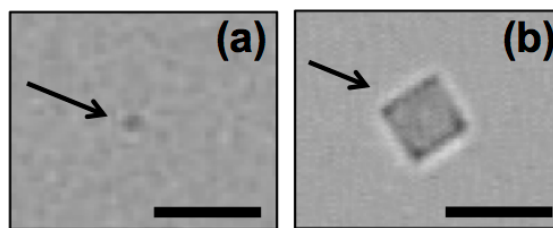


Figure 1 Laser trapping-induced *in-situ* real time Fer8 crystal formation. (a) Crystal formed by 25 min of laser irradiation and (b) the same crystal at 4 min later of the formation. Scale bar; 10 μ m.

References [1] Miura, A. et al., Appl. Phys. B, 112, 473 (2013). [2] Yoshimatsu, I et al., The 63rd JSAP Spring meeting, 2016.