# Electronic Supplementary Information (ESI) for

# Rapid localized crystallization of lysozyme by laser trapping

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## ESI 1: Estimation of local temperature elevation and temperature distribution at/around laser focus

It is considerably difficult to measure accurate temperature elevation in the laser focus with a small volume of 1 $\mu m^3$ order, but we here try to estimate the temperature elevation and distribution at/around the laser focus according to papers reported previously, because the estimation helps us to understand the effect of laser heating on crystal nucleation.

First, we roughly estimate local temperature elevation at the laser focus by referring to the experimental data reported by Ito and co-workers[1]. They successfully demonstrated the estimation of the local temperature elevation in various solvents at the focus of the trapping laser by means of fluorescence correlation spectroscopy. They reported that the local temperature elevation in the focal volume is proportional to the

laser power as the following equation, and experimentally estimated the temperature elevation at the laser focus to be 2.0 K/W in $D_2O$.

$$\frac{\Delta T}{\Delta P} \propto \frac{\alpha}{\lambda}\left(\frac{1}{r_1}\right)$$

, where $T$, $P$, $\alpha$, $r_1$, represent temperature (K), laser power (W), absorption coefficient of the solution ($m^{-1}$), thermal conductivity of solution ($Wm^{-1}K^{-1}$) and radius of the focal spot (m). On the other hand, we measured the absorption coefficient of our sample solution (HEWL buffer solution) used in this work and pure $D_2O$ at 1064 nm to be 1.6 and 0.65 $m^{-1}$, respectively. According to the above equation, since the temperature elevation at the laser focus is proportional to the absorption coefficient of solutions, the temperature elevation can be calculated to be approximately 5 K at the maximum laser power of 1.1 W in this work. Actually, the actual temperature elevation may be less than 5 K, since the numerical number of an objective lens we used (0.9) we used is smaller than that used by Ito *et al*. (1.40).

On the other hand, Celliers *et al.* in 2000 demonstrated spatially-resolved measurements of refractive index distribution induced by local heating generated in laser focus and estimate the temperature distribution around the laser focus[2]. They confirmed that the localized heating is directly proportional to laser power and the absorption coefficient of solution. According to their paper, the locally-generated temperature logarithmically decreases as a function of the distance from the laser focus and reach one half to be about 20 μm. The result strongly indicates that the temperature elevation in the area of a few mm away from the laser focus in our case cannot be considered any more. Therefore, it is reasonable to consider that the effect of laser heating on the inhibition for crystal nucleation of HEWL is excluded.

**ESI 2: Temporal change in fluorescence intensity around laser focus**

We here confirm the formation of a large highly-concentrated domain consisting of HEWL liquid-like cluster by laser trapping by measuring the temporal change in fluorescence intensity around the laser focus ($110 \times 110$ μm$^2$) for our sample used in this work. We firstly synthesized fluorescent dye-labeled HEWL (F-HEWL) by the synthetic procedure as reported previously[3], as HEWL itself shows no fluorescence. Tetramethylrhodamine-5-isothiocyanate (5-TRITC, Molecular Probes, G-isomer) with the peak of fluorescence emission intensity at 580 nm was used as the fluorescent dye. 5-TRITC was reacted to the lysine residue at N-terminal of HEWL with the ratio of 1 to 1. Since the molecular weight of 5-TRITC is considerably smaller than that of HEWL, it is reasonable to consider that there is no impact at all for the trapping dynamics and efficiency by the labelling of 5-TRITC.

The sample solution used for fluorescence measurement (HEWL, 40 mg/mL; F-HEWL, 2.5 μg/mL; NaCl, 2.7 %) was prepared by mixing 36 μL of HEWL (89 mg/mL), 2.0 μL of F-HEWL (0.1 mg/mL), 2.0 μL of sodium acetate buffer (100 mM) and 40 μL of NaCl solutions (5.4 (w/v) %), when the molar ratio of HEWL and F-HEWL is 16000:1. The prepared solution was set on an invert microscope, and then laser-trapping experiments was conducted along the same procedure as this paper. Figure S1 shows temporal change in the fluorescence intensity around the laser focus, the center of each figure ($110 \times 110$ μm$^2$). The fluorescence intensity was gradually and homogeneously increased in the whole observation area, in spite that the irradiation spot, namely the laser focus of about 1 μm$^2$, is $10^4$ times smaller than the area. After 1 hr-irradiation, the fuluorcence intensity eventually reached about twice compared to the initial solution. It is confirmed spectroscopically that laser trapping in a small volume of 1 μm$^3$ order surely

can increase HEWL concentration in the area much larger than the focus. The observation area is limited due to a field of view of the objective lens used in this experiment, however we confirm that the highly-concentrated area of HEWL extends to a few millimeter in diameter based on our experimental results in this paper.

This fluorescence measurement also reveals that short-time irradiation for 0.5 hrs increases HEWL concentration around the laser focus, although no HEWL crystallization was realized under this condition. This implies that HEWL clusters inside the domain formed by the 0.5-hrs laser irradiation are instantly diffused out because of the relatively weak interactions between molecules/clusters, and returns to the initial solution condition. Therefore, we consider that the minimum irradiation time required for triggering the densely-distributed crystal generation should be in between 0.5 to 1.0 hr.

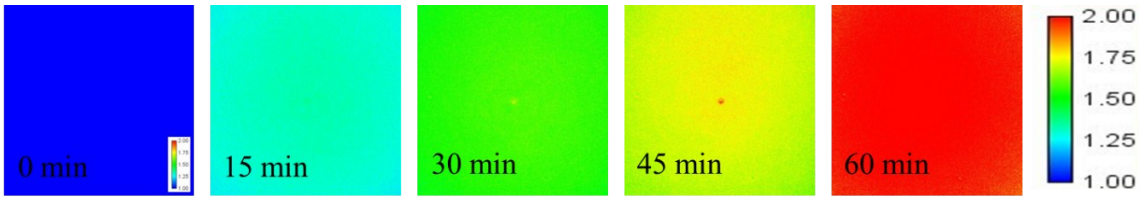


**Figure S1.** Temporal change in fluorescence intensity around laser focus, center of images. The color bar in the right side of the figure represents the ratio of the fluorescence intensity when normalized by the fluorescence intensity of initial solution (leftmost image). The color change at the laser focus is due to two-photon absorption of the trapping laser by sample solution.

REFERENCES

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