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A Microfluidic-Based Protein Crystallization Method in 10 Micrometer-Sized Crystallization Space

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Screening experiment for the seeding conditions of the PsGK crystallization

The appropriate seeding conditions for PsGK were explored to determine the best concentration of the seed solution, mixing time between the crystallization solution and the seed solution, and the mixing solution ratio. First, PsGK seed crystals were prepared by the hanging drop vapor diffusion method described above. Next, the PsGK crystals and 20 µL of reservoir solution (precipitant solution) were put into a microtube and the crushed to make a stock seed solution. The stock seed solution was diluted using the precipitant solution to an appropriate concentration. To prevent dissolution of the seed crystals, preparation of the seed solution was carried out in an ice bath.

Figure S1 shows photographs of PsGK crystals formed by the microbatch method with stock seed dilution ratios of 1:50, $1:10^2$, $1:10^3$, and $1:10^4$. Many PsGK crystals formed in all the crystallization drop. Figure S2 shows photographs of PsGK crystals formed in the microfluidic chip with 50 µm deep crystallization chambers, where the stock seed solution was diluted to $1:10^3$, $1:10^4$, $1:10^5$, and $1:10^6$. A large number of PsGK crystals formed under the high concentration seed solution condition, whereas at lower concentration, fewer crystals appeared. From these results, we consider that the dilution ratios of $1:10^5$ and $1:10^6$ are the best crystallization condition of PsGK.

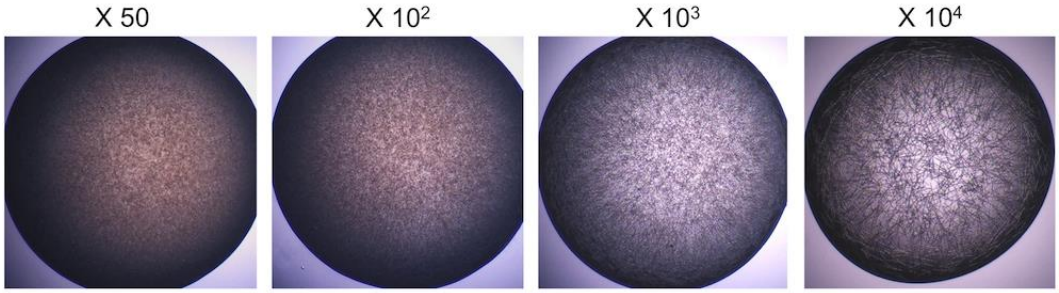

Figure S1. Photographs of PsGK seeding in the screening experiment by the microbatch method after a 24-h incubation. Dilution ratios of seed solution were 50, 10^2, 10^3, and 10^4.

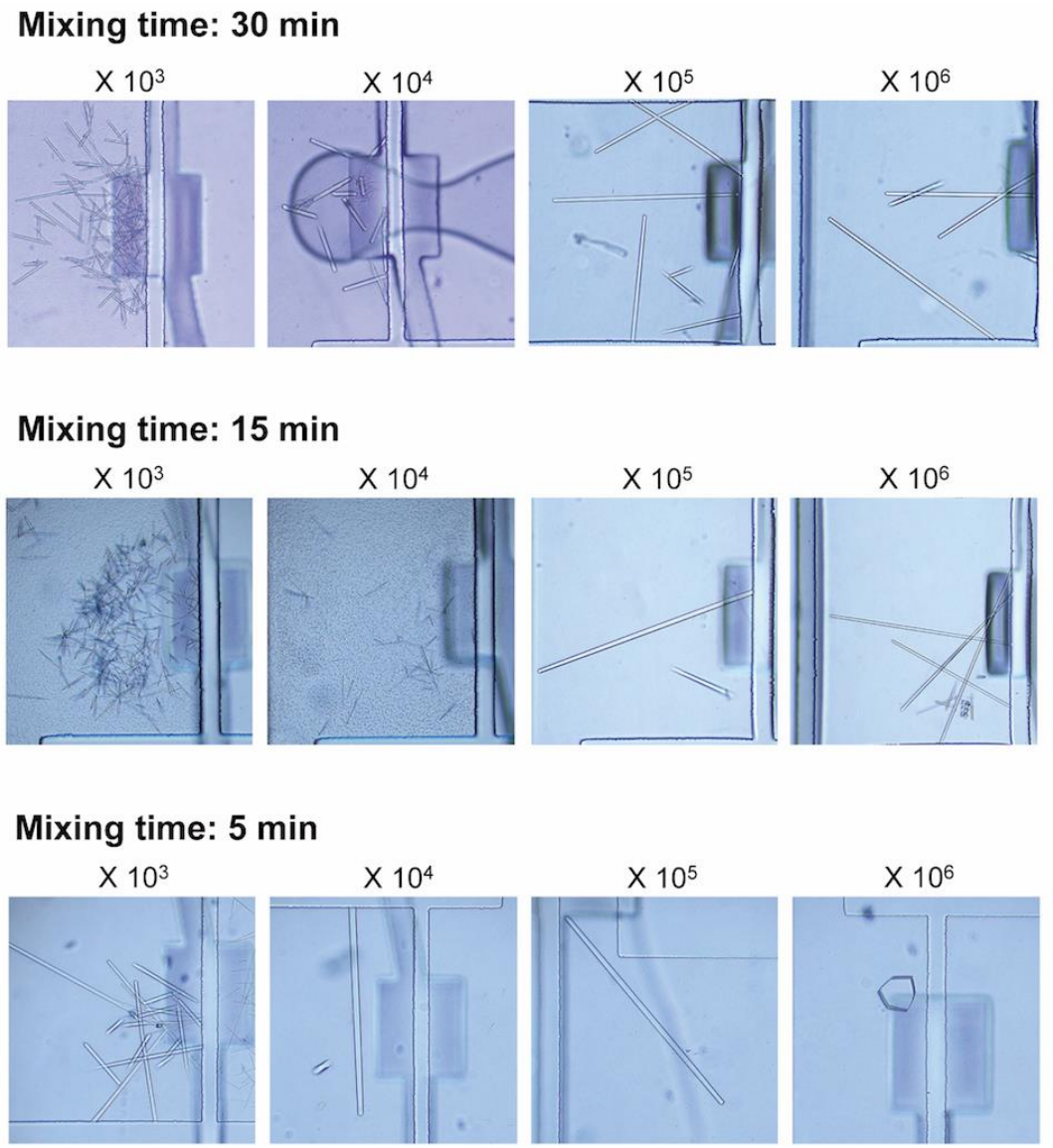

Figure S2. Photographs of PsGK seeding in the screening experiment by the microfluidic chip method after an incubation at 4°C. Dilution rates of seed solution were 10^3, 10^4, 10^5, and 10^6. The mixing time of the solutions was 30, 15, and 5 min.

Lysozyme crystal growth in the microfluidic chip with crystallization chambers of 10 μm depth

Figure S3 shows the time course of the lysozyme crystal growth in the microfluidic chip with 10 μm deep crystallization chambers. After 1-h incubation, the (1 1 0) was not oriented to the substrate, however, eventually the (1 1 0) crystal oriented parallel to the substrate was obtained.

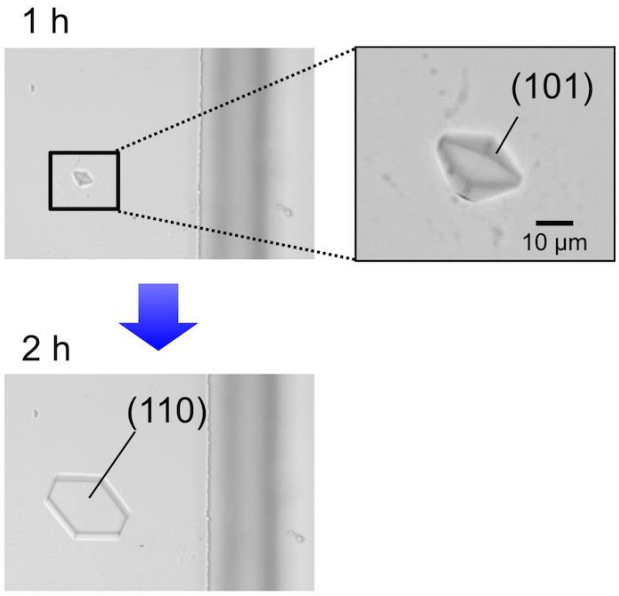

Figure S3. Time course of the lysozyme crystal growth in the microfluidic chip with 10 μm deep crystallization chambers.