Electronic Supplementary Material (ESI) for Analytical Methods.

ELECTRONIC SUPPLEMENTARY INFORMATION

Microfluidic protein crystallisation controlled using spontaneous emulsification

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S1. Microdevice cooling system

In the protein crystallisation experiments, a Peltier cooling system (Figure S1) was used to cool the protein solution, and the temperature of the copper layer was controlled by a Peltier controller (TDC-2020R). On this copper layer, an 8 cm × 3 cm scoop was prepared, into which the microdevice was set. The gap between the copper layer and microdevice was filled with ethylene glycol, and the scoop was bridged using an Al sheet in order to keep the microdevice cool.

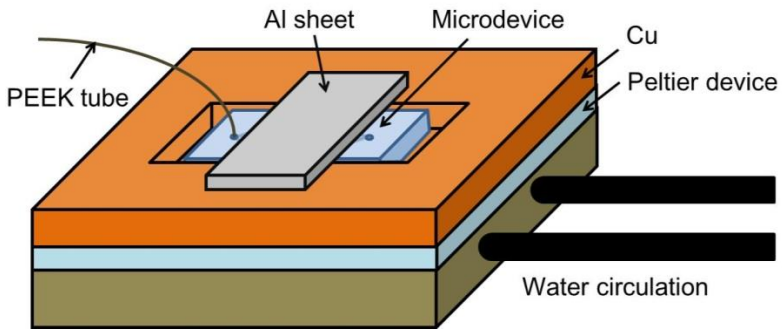

Figure S1. Cooling system for the microdevice.

S2. Calculation of water flux from the interface area and the volume of microdroplets

Here, the procedure for calculation of the water flux shown in Figure 2f, using the interface area and the volume of microdroplets is explained. It was assumed that the shape of the microdroplet was expressed as the combination of a cylinder, and of the outer half of a torus, as shown in Figure S2. The height and radius of the microdroplet were defined as r and h, respectively. Therefore, the distance from the centre to the centre of the torus, and that from the centre to the centre of the radius of the cylinder were expressed by $r - h/2$. The interface area was subsequently calculated by adding

the top (S_{top}) and the bottom (S_{bottom}) area of the cylinder with the area of the outer surface of the torus (S_{torus}) as shown in Equation S1:

$$\begin{aligned} S &= S_{top} + S_{bottom} + S_{torus} \\ &= \pi(r-h/2)^2 + \pi(r-h/2)^2 + \int_{-h/2}^{h/2} \left\{(r-h/2)+\sqrt{(h/2)^2-x^2}\right\}\sqrt{1+\frac{x}{h^2-x^2}}dx. \\ &= 2\pi(r-h/2)^2 + 2\pi h\{(r-h/2)+2h\} \end{aligned} \tag{S1}$$

In addition, the volume was calculated according to Equation S2:

$$\begin{aligned} V &= \pi\int_{-h/2}^{h/2} \left\{(r-h/2)+\sqrt{(h/2)^2-x^2}\right\}^2 dx \\ &= 2\pi h(r-h/2)^2 + \pi^2(r-h/2)h^2 + \frac{4}{3}\pi h^3 \end{aligned} \tag{S2}$$

The water flux at the time, t, after the protein solution became a microdroplet, F_t, was calculated using Equation S3:

$$F_t = \frac{-1}{\Delta t}\frac{V_{t+\Delta t} - Vt}{(S_{t+\Delta t} + S_t)/2} \tag{S3}$$

In this calculation, t and Δt were approximately 0-30 min and 5 min for 60 mM and 100 mM Span 80, respectively, and 0–120 min and 20 min for 0 mM and 30 mM Span 80, respectively. In Figure 3f, the averaged values of F_t for the nine droplets at the uppermost stream in the microdevice were plotted.

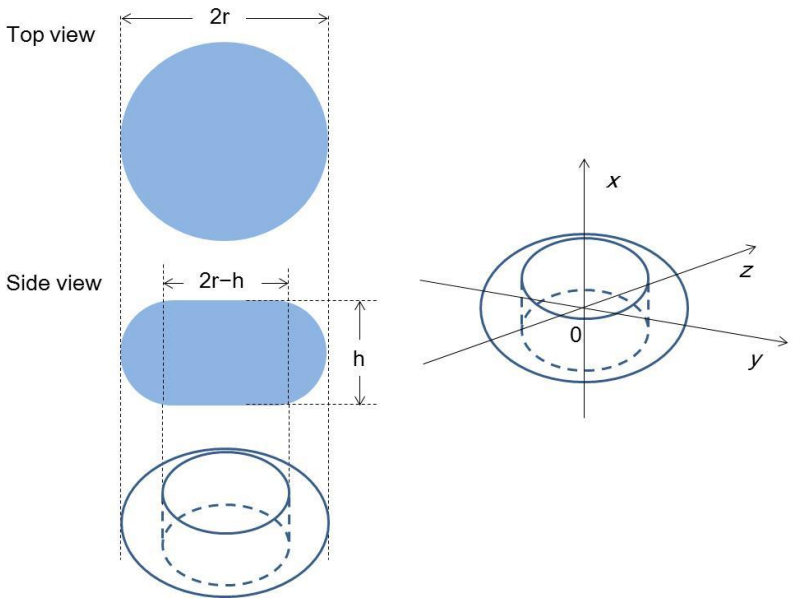

Figure S2. The assumed shape of the microdroplet.

S3. Temporal variation of microdroplets during protein crystallisation

Figure S3 shows the temporal variation of microdroplets during protein crystallisation at 33 mM Span 80. Initially, the protein solutions did not form microdroplets, as can be seen in the micrographs taken 5 min and 65 min after the protein solution was chambered. Microdroplets were observed to form gradually, as shown in the micrographs taken at 125 min and 215 min. In addition, we observed that the protein solutions began to form microdroplets earlier at higher Span 80 concentrations.

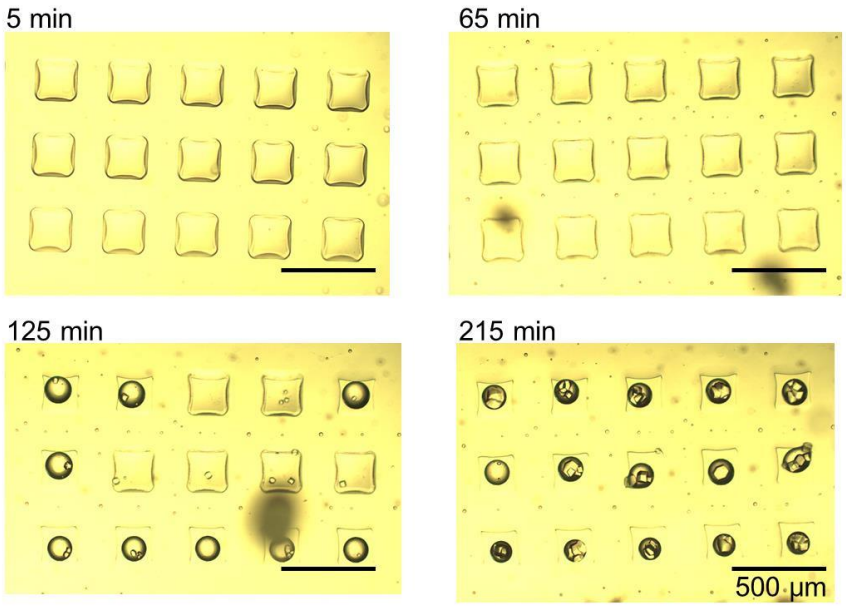

Figure S3. Temporal variation of the microdroplets (33 mM Span 80).

S4. Shape of lysozyme solutions in microwells in the lysozyme crystallisation experiments

In the lysozyme crystallisation experiments, the lysozyme solutions formed not only microdroplets, but also several other kinds of shapes. The micrographs of typical microdroplet shapes are reported below.

Type 1: Cuboid

As shown in Figure S4a, when the dodecane solution was not able to enter the gap between the lysozyme solution and the wall of the microwell, the lysozyme solution continued to fill the microwell. This behaviour was often observed at low Span 80 concentrations (0 mM and 10 mM).

Type 2: Microdroplet

When the lysozyme solution completely detached from the wall of the microwell, a microdroplet was formed, as shown in Figure S4b. This shape was commonly observed at 30 mM and 100 mM Span 80. In addition, it should be noted that in the experiments for controlling the rate of water transport by Span 80 concentration variation (Figure 3), all microdroplets were found to be of this shape.

Type 3: Partially adsorbed microdroplet

When the lysozyme solution was partially adsorbed to the wall of the microwell, a distorted microdroplet was formed, as can be seen in Figure S4c.

Type 4. Sessile droplet.

As shown in Figure S4d, a sessile droplet often formed when a microdroplet became attached to the wall of the microwell. The difference between the micrograph of a sessile droplet and that of a microdroplet was observed in the edge of the microdroplet, where the edge of the sessile droplet resembled a thin line, while that of the microdroplet resembled a thick line, due to the reflection of light at the interface of the microdroplet.

In order to obtain a distribution of the numbers of crystal in each microwell using a number of Span 80 concentrations (Figure 5), we counted the number of crystals in all microwells in the device regardless of the shape of the lysozyme solutions.

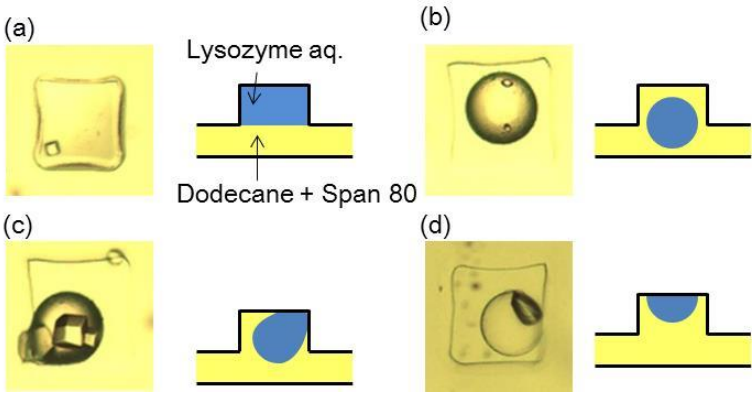

Figure S4. Micrographs of typical shapes of lysozyme solutions. (a) Cuboid; (b) Microdroplet; (c) Partially adsorbed microdroplet; and (d) Sessile droplet.