Electronic Supplementary Information

High-throughput sample introduction for droplet-based screening with an on-chip integrated sampling probe and slotted-vial array†

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Chemicals and reagents

N-tetradecane (Acros Organics) was used as oil carrier in the droplet system. In the screening of protein crystallization condition, thaumatin (22.2 KDa, Sigma-Aldrich) was used as model protein, and 30 mg/mL thaumatin solution was prepared with 0.1 M buffer solution (pH=6.5) of N-(2-acetamido) iminodiacetic acid (ADA, Sigma-Aldrich). Six precipitants including 2.0 M Potassium sodium tartrate tetrahydrate in 0.1 M HEPES sodium salt (pH=7.5), and No. 4, 16, 32, 33, 35 precipitants in Crystal Screen reagent kit (HR2-110, Hampton Research) were used, their compositions are shown in Table S1. All the solutions were prepared fresh before use.

Table S1 Compositions of the screened precipitants.

No.	Precipitants
1	2.0 M Ammonium sulfate, 0.1 M TRIS hydrochloride pH= 8.5
2	1.5 M Lithium sulfate monohydrate, 0.1 M N-(2-hydroxyethyl) piperazine-N'-ethanesulfonic acid (HEPES) sodium salt, pH=7.5
3	2.0 M Ammonium sulfate
4	4.0 M Sodium formate
5	0.8 M Sodium phosphate monobasic monohydrate; 0.8 M Potassium phosphate monobasic, 0.1 M HEPES sodium salt, pH=7.5
6	2.0 M Potassium sodium tartrate tetrahydrate, 0.1 M HEPES sodium salt, pH=7.5

Fabrication of microchip

The present droplet-based screening system consisted of the BEE chip, SVA sample presenting system, and two syringe pumps (PicoPlus 11, Harvard Apparatus, USA) (Fig. 1a). The channel configuration of the BEE chip is illustrated in Fig. 1c. The chip was fabricated of glass plates using standard photolithographic, wet chemical etching and high-temperature bonding techniques as described elsewhere.[22] The photo mask design of the BEE chip is as shown in Fig. S1. The etched sample introduction, reagent merging and ventral channel had the same width of 110 μm and depth of 20 μm. The on-chip monolithic sampling probe was fabricated as reported previously by the authors' group.[23,24] Briefly, an emery drill installed in the bench drill and sand papers were used to gradually grind the glass chip to fabricate a cone-shaped sampling probe. The tip size of the probe was ca. 210 × 60 μm (width × thickness) (Fig 1b,d and Fig. S2). The outer surface of the sampling probe was further polished using polishing powder. Before use, the outer surface of the sampling probe and all of the chip channels were silanized with 4 mM octadecyl-trichlorosilane (Acros Organics) in n-heptane for 4 h at 25 °C.

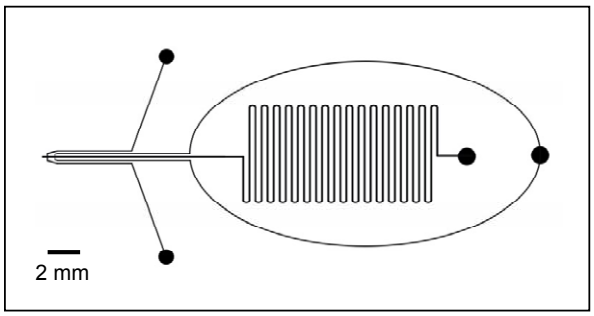

Fig. S1 The photo mask design of the BEE chip.

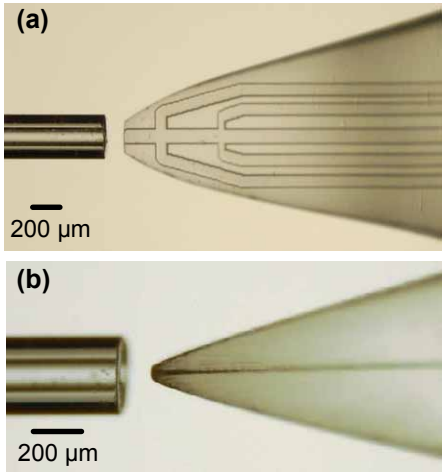

Fig. S2 (a) Top view and (b) side view of the integrated sampling probe with a fused-silica capillary (150 μm i.d., 320 μm o.d.) for comparison.

Droplets observation

A stereoscopic microscope (SMZ1500, Nikon, Japan) and a high-speed camera (Phantom Miro3, Vision Research Inc., USA) with a highest frequency of 1000 fps were used to observe and record the droplet generation process. An inverted microscope (ECLIPSE TE-2000-S, Nikon, Japan) coupled with a CCD camera (SPOT RT-SE6 Monochrome, Diagnostic Instruments, USA) was used for protein crystal observation.

Experiment for high throughput sample introduction

A limit experiment was performed using almost the ultimate withdrawal rate of 9 μL/min in the present system to test the possible highest sampling throughput of BEE for different samples. A droplet generation frequency of ~24 Hz was obtained, and the throughput for different samples reached to 6600 h^{-1} with generating 13 droplets for each sample. The total sample introduction time for each sample was 0.54 s, including the sampling time of the probe of 0.39 s, and the SVA switching time of 0.15 s. Figure S3 shows a series of images of the sample introduction process recorded by the high-speed camera.

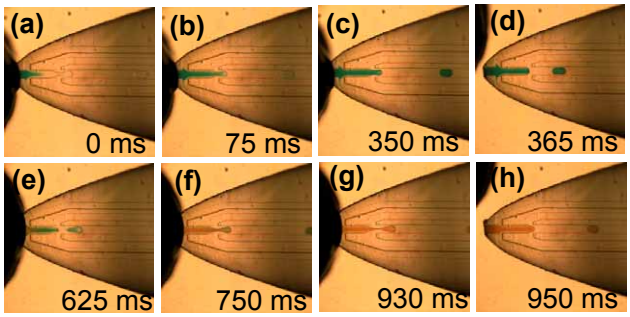

Fig. S3 Images of the sample introduction process recorded by the high-speed camera. (a) The blue dye in the vial begins to be aspirated into the sampling probe to replace the previously introduced water. (b) Pure blue dye droplets begin to be generated. (c) The blue dye vial begins to remove from the sampling probe. (d) The sampling probe is exposed in air. The sampling for blue dye is completed. (e) The brown dye vial is switched to the probe, and the brown dye begins to be aspirated into the probe to replace the blue dye. (f) Pure brown dye droplets begin to be generated. (g) The brown vial begins to remove from sampling probe. (h) The sampling probe is exposed in air. The sampling for brown dye is completed.