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Supporting Information

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A Microfluidic Approach for Screening Sub-Microliter Volumes Against Multiple Reagents Using Pre-formed Arrays of Nanoliter Plugs in a Three-Phase Liquid/Liquid/Gas Flow

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Preparation and transportation of an array of plugs:

First a small piece of Teflon tubing (outer diameter 250 μm, inner diameter 200 μm) was connected to a 10 μL syringe (Hamilton). The syringe and the Teflon tubing were filled with fluorocarbon as carrier fluid. The fluorocarbon is a mixture of perfluoroperhydrophenanthrene (PFP) and 1H,1H,2H,2H-perfluorooctanol (PFO) at volume ratio of 10:1. To prepare an array of plugs, different reagents, fluorocarbon, and air bubbles were aspirated successively into a piece of small Teflon tubing. This is achieved by pulling the plunger of the syringe that was connected with the Teflon tubing while the other end of the Teflon tubing was in the corresponding solution. During the aspiration process, the movement of the plunger was controlled by a manual micrometer syringe driver (Stoelting Co.). Before the aspiration of each reagent, a small amount of fluorocarbon was aspirated into the Teflon tubing to separate the plugs. In most experiments, an air bubble was also aspirated into the Teflon tubing after the fluorocarbon aspiration. After the array of the plugs was prepared inside the Teflon tubing, more fluorocarbon was aspirated into the tubing to make sure all the plugs (and air bubbles) were surrounded by fluorocarbon. The Teflon tubing was then inserted into a funnel-shaped capillary (Hampton Research). By pushing the plunger of the syringe that was connected to the Teflon tubing, the array of the plugs was transferred into the capillary (OD 0.20 mm, ID 0.18 mm). The capillary

was then cut off from the tubing and sealed by wax for long-term storage. To utilize the array for experiments in microfluidic channels, it was first transported from the capillary into the Teflon tubing. The Teflon tubing was then inserted into a funnel-shaped adapter that was coupled to the inlet of the microfluidic channel. The transportation of the array was controlled by the syringe that was connected to the Teflon tubing. At the same time, a stream of the target solution was injected into the side channel and this stream merged with the array of the plugs, mixed, and the reaction occurred. The array of the plugs after mixing was transported into a capillary using a similar funnel-shaped adapter. After the capillary was filled with the array of the plugs, the capillary could be taken off the microfluidic channel and sealed for incubation or analysis.

Functional assay of enzymes against one substrate:

An array of plugs was prepared, which contained alkaline phosphatase (AP) (0.02 mg/ml in 0.2 M diethanol amine, pH 10.5), catalase (0.02 mg/ml in PBS, pH 7.3), ribonuclease A (RNase) (0.02 mg/ml in 0.05 M Tris buffer, pH 7.5), and lysozyme (0.02 mg/ml in 0.05 M NaAc buffer, pH 4.5). The array had one plug of each enzyme, and every two neighboring enzyme plugs were separated by two plugs of PBS buffer. An air bubble was inserted between every two neighboring aqueous plugs. To assay the activity of the four enzymes on the substrate fluorescein diphosphate (FDP) (11 μM with 0.5 M NaCl), the array of plugs was merged with the solution of FDP at a T-junction. The flow rates of the array and the FDP stream were 1.2 μL/min and 0.5 μL/min, respectively. The array of the merged plugs was collected in a capillary and the fluorescence image of each plug was taken by a fluorescence microscope (Leica

DMIRE2) equipped with a digital camera (Hamamatsu, ORCA-ER). Fluorescence intensity in the images was analyzed using Metamorph Imaging System (Universal Imaging).

Screening precipitants for crystallization conditions of thaumatin:

To screen the 48 precipitants from the *Crystal Screen* kit (Hampton Research), an array of 48 plugs of 48 precipitants (from No. 1 to No.48) was prepared. The reagent formulation of the precipitants can be found at the website of Hampton Research (http://www.hamptonresearch.com/support/guides/2110F.pdf). The precipitant reagents had various salt concentrations (e.g., No. 44 0.2 M ammonium formate and No. 33 4.0 M sodium formate) and various viscosities (e.g., No. 30, 30 % w/v PEG 8000). Every two neighboring plugs were separated by an air bubble. The carrier fluid was the mixture of PFP and PFO (volume ratio 10:1). After the array was prepared, it was transported into the microchannel for screening. Another piece of Teflon tubing was connected to a 10 μL syringe and the syringe + tubing assembly was filled with PFP. Slightly less than 1.0 μL solution of thaumatin (60 mg/mL in 0.1 M N-(2-acetamido)iminodiacetic acid buffer, pH 6.5) was aspirated into the tubing. The tubing was then inserted into the inlet of the side microchannel of the T-junction. The solution of thaumatin was driven into the main channel and merged with the array of the plugs of precipitants. The flow rates of the thaumatin solution and the array were 1.2 μL/min and 0.5 μL/min, respectively. After the plugs were merged with thaumatin solution, they were collected in a capillary and sealed by wax for incubation. Less than ~0.1 μL of thaumatin solution remained in the channel. This method does not require large volumes of solution and does not generate much waste, and is useful with volumes as low as sub-microliter, screened against nanoliter plugs of multiple reagents.

To screen the five precipitants from the *Crystal Screen* kit, an array of plugs was prepared, which contained five different precipitants from the screening kit: No.13 (0.2 M sodium citrate/0.1 M tris HCl/30 % v/v PEG 400, pH 8.5), No.24 (0.2 M $CaCl_2$/0.1 M NαAχ/20 % ϖ/ϖ ισοπροπανολ, πΗ 4.6), No.25 (0.1 M ιμιδαζολε/1.0 M NαAχ, πΗ 6.5), No.29 (0.1 M ΗΕΠΕΣ/0.8 M ποταϲϲιυμ σοδιυμ ταρτρατε, πΗ 7.5), ανδ No.33 (4.0 M σοδιυμ φορματε). Τηε αρραψ ηαδ τωο πλυγσ οφ εαχη πρεχιπιταντ ανδ τοταλλψ τεν π λυγσ. Αν αιρ βυββλε ωασ ινσερτεδ βετωεεν εϖερψ τωο νειγηβορινγ πλυγσ. Τηε μιξτυρε οφ ΠΦΠ ανδ ΠΦΟ (ϖολυμε ρατιο 10:1) ωασ υσεδ ασ τηε χαρριερ φλυιδ. Αν αθυεουσ σ τρεαμ οφ τηαυματιν (~ 60 μγ/μλ ιν 0.1 M ΑΔΑ βυφφερ, πΗ 6.5) ωασ ινφεχτεδ ιντο τηε αρ ραψ οφ τηε πλυγσ φορ χρψσταλλιζατιον. Τηε φλοω ρατεσ οφ τηε αρραψ ανδ τηε στρεα μ οφ τηαυματιν ωερε 1.2 μL/min and 0.5 μL/min, respectively. Incubation of the plugs at 18°C resulted in crystallization of thaumatin in plugs that contained precipitant No. 29 and thaumatin.