Control of Concentration and Volume Gradients in Microfluidic Droplet Arrays for Protein Crystallization Screening

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Abstract—**A novel screening platform for the screening and optimization of protein crystallization is reported. Here we present two key experiments to generate protein crystallization phase diagrams. One is to precisely generate spatial gradient droplet arrays; the other is to trap and identify each droplet in place in order to carry out temporal and spatial analysis. The generated concentration and volume gradient ranges from 0% to 100% and from 155 to 310 pico liters, respectively. Furthermore, the active trapping of a droplet array in a microfluidic chip for hours to days to study the dynamic phase changes in protein crystallization is demonstrated. This microfluidic platform can be used for rapidly generating the solubility diagram for protein in various salt solutions.**

Keywords—**Microfluidics, droplet arrays, screenings**

I. Introduction

Previous work on microfluidic droplet generation has shown promise for the development of high throughput screening assays [1-4]. These microfluidic systems are significant in that they are able to use a very small quantity of sample solutions (pL to nL) for each combinatorial test in droplets. However, screening based on PDMS microfluidic chips to obtain the solubility of protein with "accuracy" still face numerous challenges such as the transport of viscous solutions [5] and the trapping of solution droplets in microfluidic channels without droplet coalescence. Stabilization methods include polymerization and the addition of surfactants to the droplet may avoid droplet fusion, but these may ultimately affect the evaporation rate and the chemical content of the droplet. Other significant operation platforms, for example, manual screening based on the robotic system is expensive [6] and screening in robust microfluidic device is not yet widely used in individual laboratory [2]. In this paper, we demonstrate a platform for the dynamic control of concentration and volume gradients in microfluidic droplets and for the active trapping for temporal and spatial analysis of each droplet trapped in place.

II. Experimental

1) *Operation System Details*:

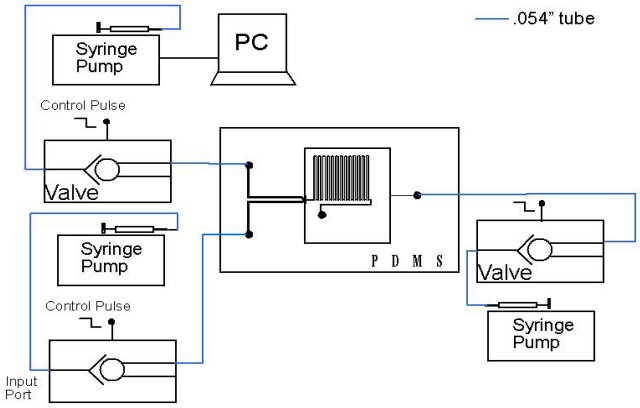

Fig 1. Experimental setup

The three fluids are injected into the PDMS channel by syringe pumps (*Pico Plus*, Harvard Apparatus) programmed by an RS232 interface. A zero-dead-volume valve from the Lee Company is connected in between the chip inlet and the pump to actively trap the droplet arrays [7]. The control pulses applied to these valves are in phase with each other so that the valves are synchronized.

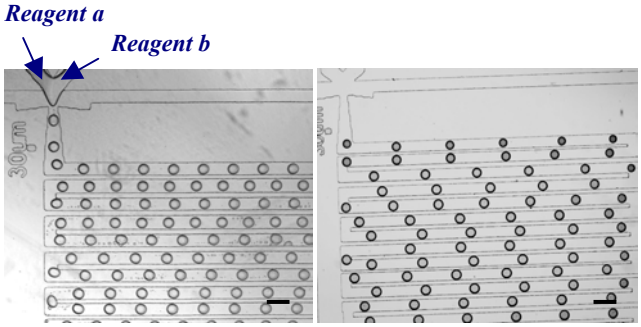

Fig 2. A Droplet array carrying two reagents in flow (A) and trapped (B) in microfluidic channels. (Scale bar, 100μm)

2) Fabrication of Microfluidic Chip: The fluidic channels are fabricated by molding PDMS (10:1 polymer to curing reagent) to a SU-8 mode. After cured, the PDMS and a clean glass slide are treated with one minute oxygen plasma (Harrick Scientific Co.) at high power to be surface bonded. The microfluidic channels demonstrated in this paper are with a height of 50 μm.

3) Microfluidic Channel Design:

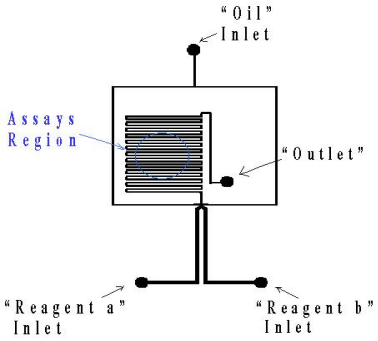

Fig 2. Schematics of microfluidic chip.

Symmetric end-pinching microfluidic channels were used to generate droplet emulsions [1]. The 3D microfluidic channels geometry may be designed to avoid the concentrated viscous solutions from attaching to the channel surface.

4) Liquid Preparation and Operation: Droplet may be generated from a variety of immiscible fluids. "Olive oil" is used as the continuous phase, and "reagent a" and "reagent b" are mixed and treated as the dispersed phase, as shown in Fig. 2. Here olive oil is selected due to its better sealing property to prevent the water evaporation of the droplets. Reagent a contains 65mg/mL lysozyme protein in 0.1 M pH 4.8 NaAcetate, 0.02% Na-Azide, and fluorescein isothiocyanate-dextran (FITC-Dextran); Reagent b contains 1.0 M NaCl in 0.1 M pH 4.8 NaAcetate, 0.02% Na-Azide, and fluorescein TAMRA.

III. Results

A. Generation of Concentration Gradients

The concentration of the solution droplet varies with the flow rate of one of the reagents. For example, as the flow rate of reagent b increases, the concentration of reagent b in the droplet increases. A concentration gradient droplet array is ramped from 0% to 100%, as shown in Fig 3a. In this experiment, the flow rate of "reagent b" is programmed by an RS232 interface, and the flow rates of "reagent a" and "oil" remain constant.

After trapping a gradient droplet array, the fluorescence intensity of each droplet can be measured by using the imaging program (MetaMorph 6.0) to acquire the ratio between the reagents. A fluorescence combination should be chosen to avoid overlapped excitation wavelengths, as shown in Fig 3b, so that the two reagents can be distinguished through a filter.

A good feature of this dynamic controlled concentration gradient is the ability to manipulate a large set of picoliter droplet arrays without altering the droplet size.

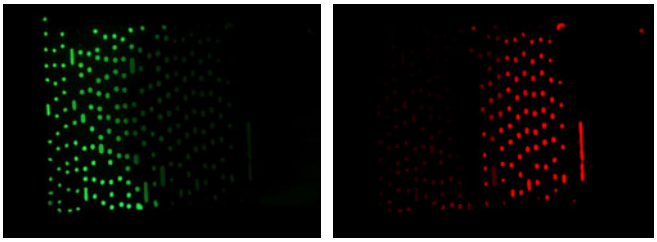

Fig 3a. Fluorescence micrographs show the control of concentration gradients in microfluidic droplet arrays. "Reagent a" (protein solution) is labeled with green fluorescent dye (FITC-Dextran); "reagent b" (salt solution) is labeled with red fluorescent dye (TAMRA). Fluorescence micrographs of the microfluidic chip were taken by an inverted microscope (Nikon, NY) with a 10X objective and a CCD camera.

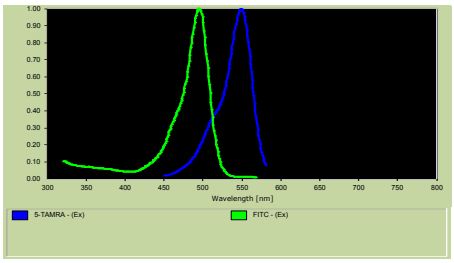

Fig 3b. The fluorescence excitation plot of two fluorescent dyes, FITC-Dextran and TAMRA, demonstrated in Fig 3a.

B. Generation of Droplet Volume Gradients

An increase in shear force applied on the water surface results in a decrease in droplet volume as shown in Fig 4. The magnitude of the viscous stress exerted by the oil system can be controlled by changing the oil flow rate. In this experiment, the oil flow rate is ramped from 0.4~2.4 μl/min by programming the syringe pump through an RS232 interface. The images are recorded with a high-speed camera with a frame rate of 500 frames per second. The sizes of the droplets are measured by area pixels of droplets on gray scale using the imaging program (*Scion Imag*).

Theoretically the droplet size can be predicted by equating the Capillary number defined as the balance of interfacial tension and shear force [1]:

$$Ca = \frac{\eta_c \gamma \left(\frac{D}{2} \right)}{\sigma}$$

Where D is the diameter of the droplet, σ is the interfacial tension between water and oil phase, η is the viscosity of the continuous phase, and γ is the shear rate. Droplet breakup occurs when $Ca > Ca_{critical}$.

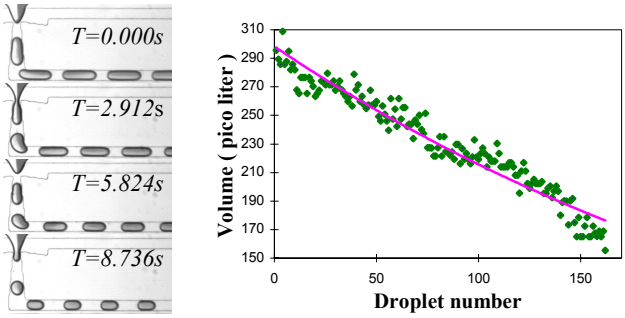

Fig 4. Control of volume gradients in a microfluidic droplet array. 162 droplets are generated with the droplet volume from 155 to 310 pico liters in 8.736s.

The ability to successively vary the sizes of the droplets is critical for nucleation and solubility assays based on the diffusion rate of the reagent droplets through the microchannels. This is a better platform compared to hanging drop methods because the diffusion can be achieved without contaminating the sample drops open to the air [8, 9]. In addition, the smaller droplet may have a higher quality crystal growth.

C. Screenings of Protein Crystallization Conditions

A preliminary experiment is demonstrated for screening of protein crystallization conditions by observing lysozyme solubility in droplets. We observed protein crystallization by controlling the concentration gradients in a droplet array on a microfluidic chip, as shown in Fig 5a, 5b. In this experiment, the droplet array contains the droplets with the "reagent a" (salt solution) from 0% to100% and the "reagent b" (protein solution) from 100% to 0%. After trapping As seen in Fig 5b, three phases were obtained in a microfluidic droplet array: 1) protein supersaturated state inside the droplets generated with high protein concentration, 2) protein crystallization inside the droplets with salt and protein solution ratio around 1:1, 3) salt crystallization inside the droplets generated with high salt concentration.

After trapping, the microfluidic channel openings were sealed with PDMS and kept the chip in 20 °C. However, additional crystallization solubility parameters such as temperature, pH, and ionic strength can also be generated based on this system to provide multidimensional phase diagrams. Temperature may be varied to explore ways that it may be used to move protein solutions into supersaturated zones and to affect crystallization process. Therefore, temperature should be a factor in each of the models describing concentration, pH, and salinity control [10]. The ability to arbitrarily generate solubility diagrams for proteins will enable rapid protein crystallization. Therefore, this system that is capable generating gradient/volume droplet arrays that are trapped in place has the potential to be a powerful platform to obtain the optimal parameters for crystal growth.

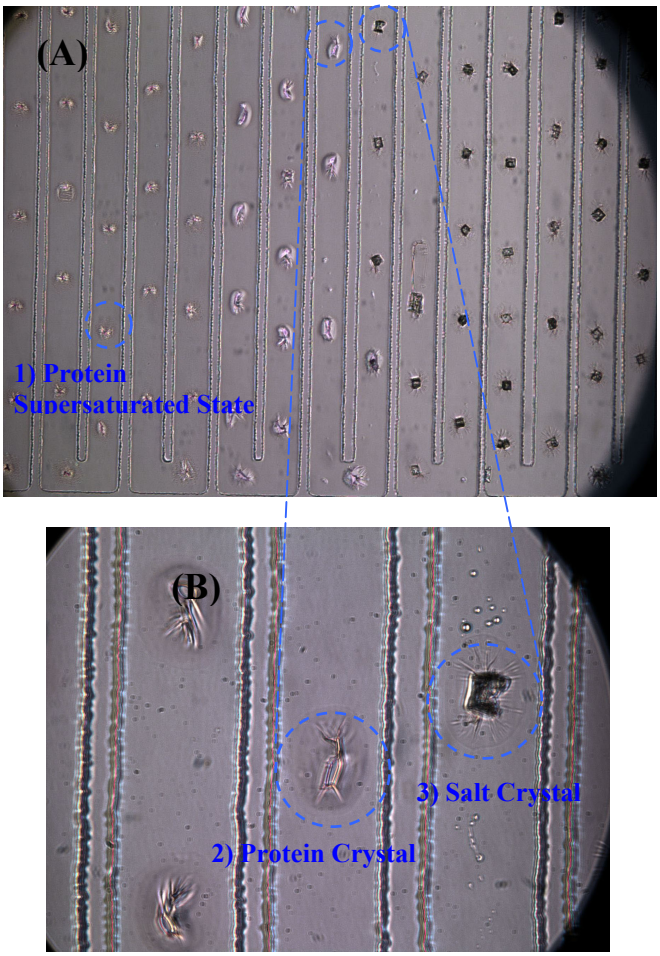

Fig. 5. Optical micrographs of protein crystallization observed in a gradient droplet array in chip. Images were taken by an inverted microscope (Nikon, NY) with a 10X objective (A) and a 40X objective (B). (Scale bar, 100μm)

IV. Discussion

In order to adapt this platform to the other solubility assays (temperature, pH, and ionic strength), there are a few parameters and processes that need to be taken into consideration for the generation of droplet arrays. These include the pumping of high viscosity fluids in microfluidic channels, the thorough hydrophobic treatment of microfluidic channels, and the minimization of fluidic resistance of the microchannels while maintaining sufficient shear forces. Future work will involve the quantification of droplet solution evaporation rates, and the generation of many more gradients to plot the solubility diagrams.

V. Conclusion

The experimental results presented in this paper have demonstrated the ability to reconfigure and control the generation of concentration and volume gradient droplet arrays. This advanced platform is easy to fabricate, setup, and program to generate a large set of gradient droplet arrays with applications in the synthesis of molecular drugs (genes, proteins, etc.), combinatorial chemical assays, and the analysis of biochemistry reactions.

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