Establishment of biochemical high-throughput screening systems in microfluidic emulsion

Ph.D. Dissertation (Summary)

マイクロフルイディックエマルジョン中でのハイスループット生化学反応スクリーニングシステムの開発（要約）

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INTRODUCTION

Main focus of this study was done on developing microfluidic emulsification system capable of producing emulsion for different biochemical applications. Among the many existing microfluidic system only few have characteristics suitable for emulsified reactions such as emulsion PCR and *in vitro* transcription/translation, and a lot of optimization steps is required for each screening system. For example, monodisperse emulsion can be produced by microchip devices, but the emulsification time is prohibitive for the applications requiring usage of microbeads or cell-free protein synthesis. Also, it is more difficult to produce monodisperse emulsion of small diameter that large, but it is important for increasing number of droplets per ml of emulsion. Moreover, droplet diameter greatly affects heat stability of the emulsion, with smaller droplets having better stability than the larger ones.

Here an attempt was made to develop universal microbead display for promoter screening. To achieve this goal several designs of microfluidic nozzles were proposed and configuration was optimized for different oil phase compositions as well as different biochemical reaction mixtures. In addition, microbead display was proposed for promoter screening using GFP reporter expressed by cell-free protein synthesis system compartmentalized in emulsion and selection by fluorescence-activated droplet sorting.

MAIN RESULTS

A simple, inexpensive flow-focusing device has been developed to make uniform droplets for biochemical reactions, such as *in vitro* transcription and cell-free protein synthesis (Figure 1). The device was fabricated from commercially available components without special equipment. Using the emulsion droplets formed by the device, a class I ligase ribozyme, bcI 23, was successfully synthesized from DNA attached to magnetic microbeads by T7 RNA polymerase (Figure 2). It was also ligated with an RNA substrate on the same microbeads, and detected using flow cytometry with a fluorescent probe. In addition, a single-chain derivative of the lambda Cro protein was expressed using an *Escherichia coli* cell-free protein synthesis system in emulsion, which was prepared using the flow-focusing device (Figure 3). In both emulsified reactions, usage of the flow-focusing device was able to greatly reduce the coefficient of variation for the amount of RNA or protein displayed on the microbeads, demonstrating the device is advantageous for quantitative analysis in high-throughput screening. Later on, a universal microbead display for promoter screening using sfGFP reporter expressed by cell-free protein synthesis system compartmentalized in emulsion and selection by fluorescence-activated droplet sorting was proposed. This system was tested on 1:100 model library and high enrichment was observed in a single round of screening (Figure 4).

FIGURES

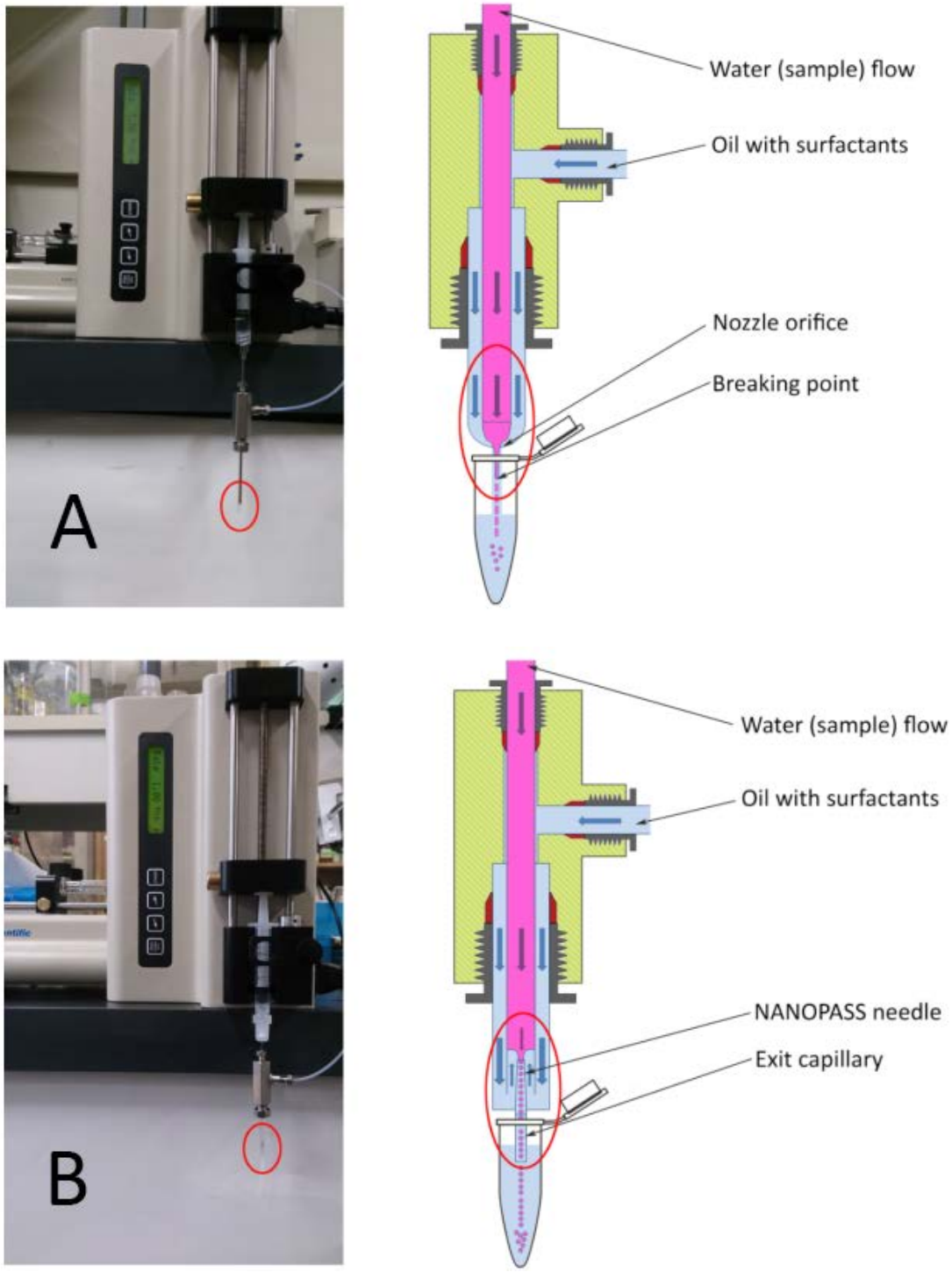
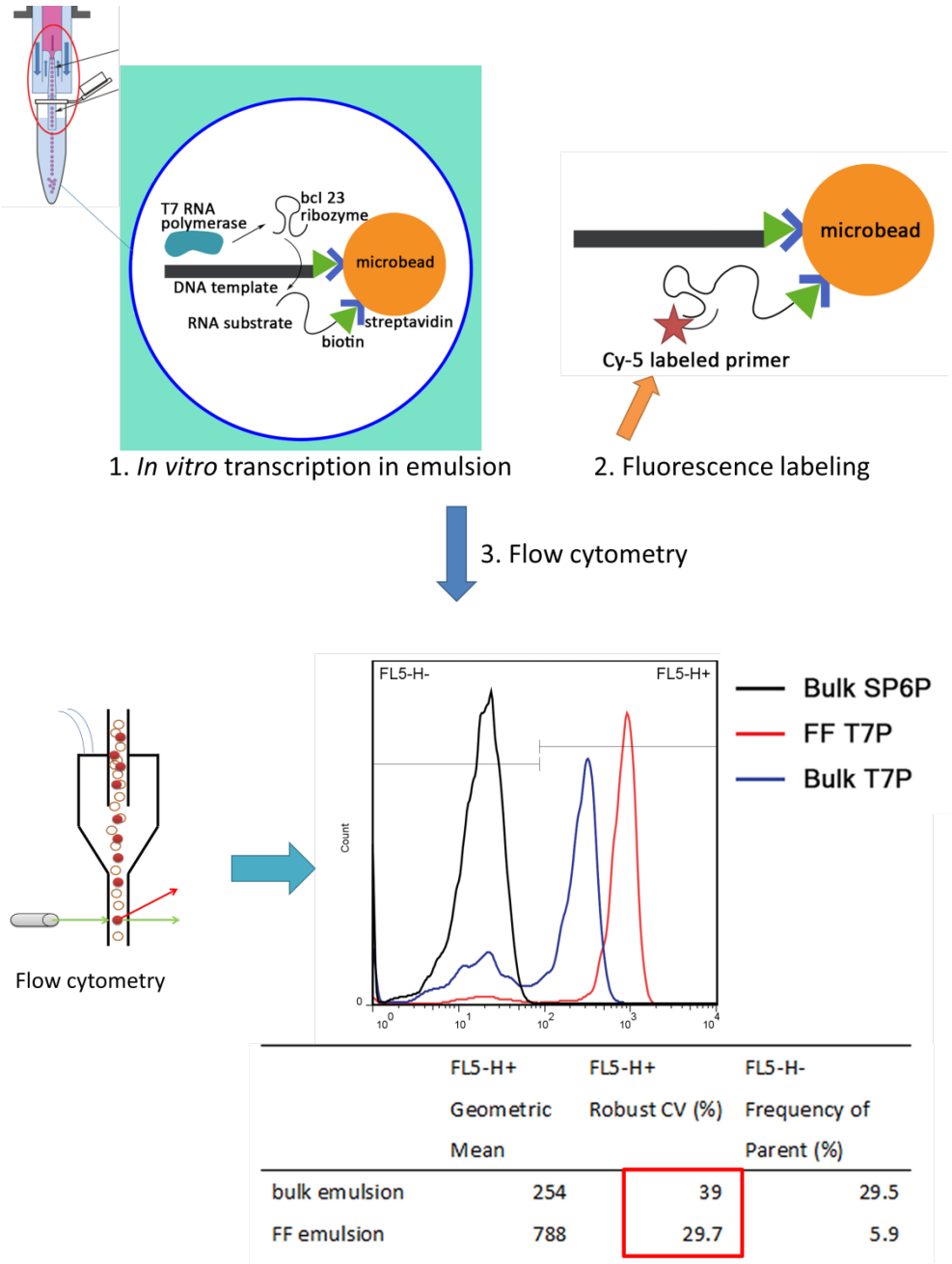
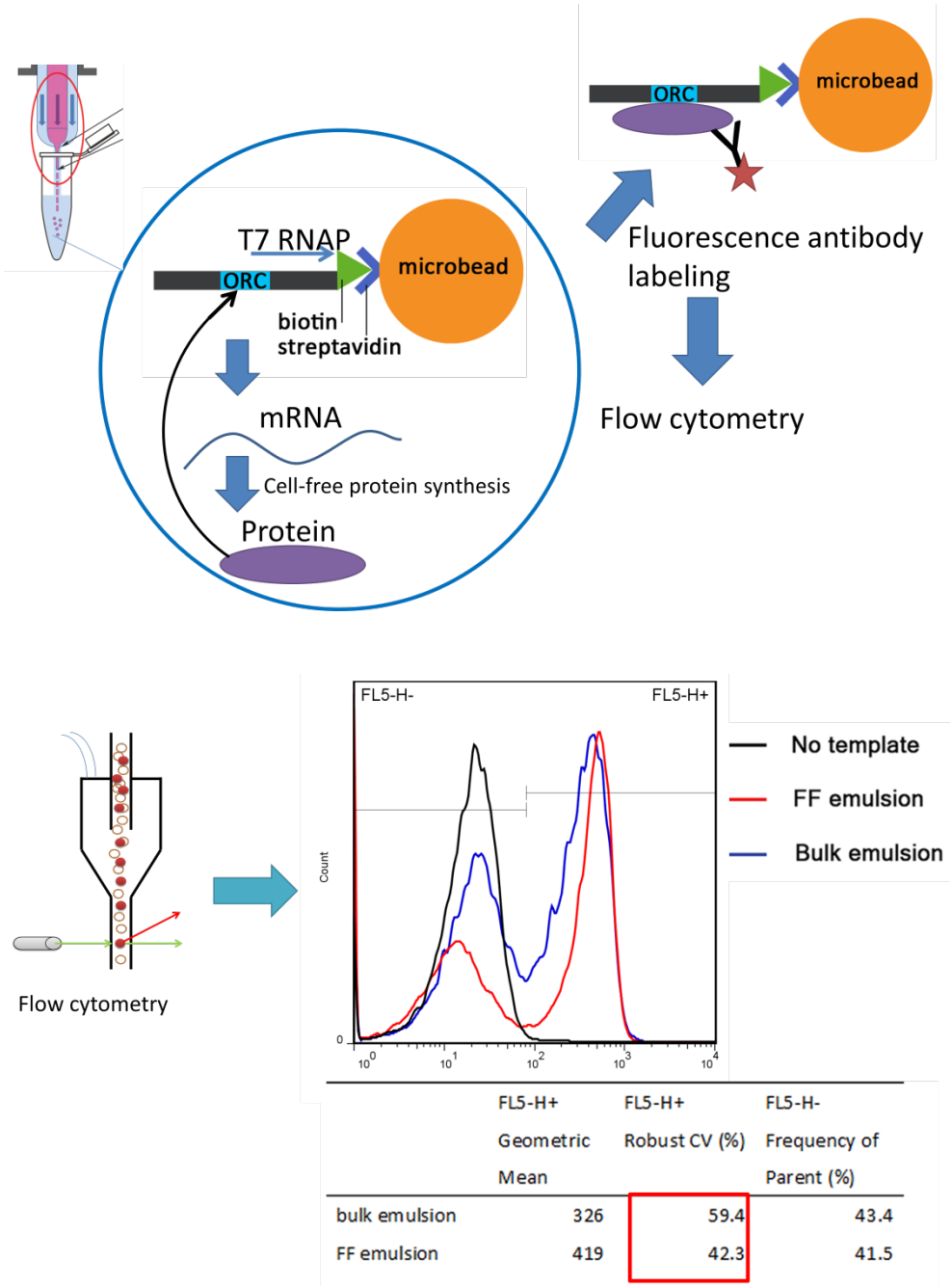


Figure 1. Schematics of flow-focusing devices: A. jet collection device; B. capillary collection device. Red circles indicate droplet generation area.



	FL5-H+ Geometric Mean	FL5-H+ Robust CV (%)	FL5-H- Frequency of Parent (%)
bulk emulsion	254	39	29.5
FF emulsion	788	29.7	5.9

Figure 2. *In vitro* transcription of bcI 23 ribozyme in emulsion produced by capillary collection device.



	FL5-H+ Geometric Mean	FL5-H+ Robust CV (%)	FL5-H- Frequency of Parent (%)
bulk emulsion	326	59.4	43.4
FF emulsion	419	42.3	41.5

Figure 3. Cell-free protein synthesis in emulsion produced by jet collection device.

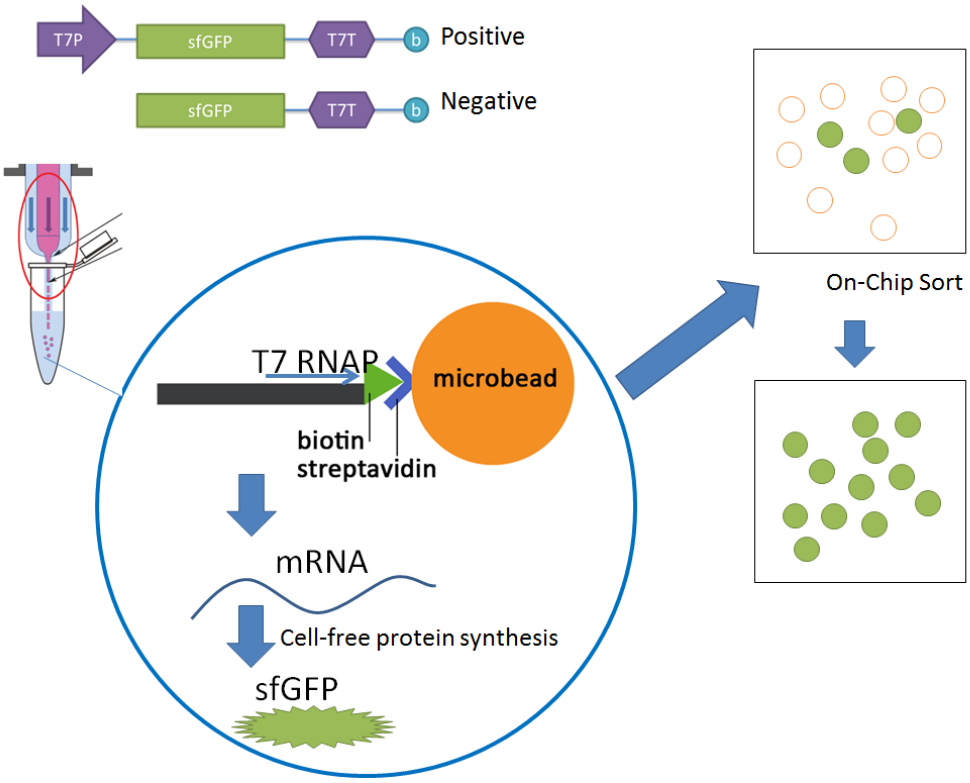


Figure 4. Microbead display for promoter screening using sfGFP reporter expressed by cell-free protein synthesis system compartmentalized in emulsion and selection by fluorescence-activated droplet sorting (scheme of experiment)