HIGH-THROUGHPUT ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS IN DROPLET-BASED MICROFLUIDICS USING FLUORESCENCE POLARIZATION

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ABSTRACT

In recent years droplet-based (or segmented-flow) microfluidic systems have emerged as a powerful technological platform for performing high-throughput chemical and biological experimentation. Herein, we have demonstrated the combination of fluorescence polarization and droplet-based microfluidics for the rapid analysis of protein-protein interactions. Fluorescence polarization is powerful technique for analysis of biomolecular interaction since it is truly homogenous assay format. In this study, specifically, the interaction between angiogenin and anti-angiogenin antibody was successfully analyzed in short times of 40 hertz and with high precision. Angiogenin is a small polypeptide implicated in angiogenesis and in tumor growth.

KEYWORDS

Droplet-based microfluidics, Protein-protein interaction, Fluorescence polarization

INTRODUCTION

Droplet-based microfluidic systems have emerged as a useful technology in academic fields of biochemistry and molecular biology. Also, it is regarded as a suitable platform for high-throughput screening experiments because a complete mixing is achieved within milliseconds and each droplet can be used as a individual micro-reactor with pico-liter scale. Recently, various detection detection methods for analysis of bio-molecular interactions have been developed in droplet-based microfluidic system. Especially, fluorescence resonance energy transfer (FRET) is most widely used. In case of biological screening, however, because FRET is needed multiple labeling steps it is labor-intensive, time consuming and costly.

Fluorescence polarization is based on the principle of photoselective excitation of a population of fluorophores using polarized radiation. In simple terms, fluorophores with dipoles parallel to the excitation radiation will absorb photons, whereas those with dipoles perpendicular to excitation radiation will not. This leads to partially polarized fluorescence emission. In free solution, the observed emission will depolarized via rotational diffusion. This rotational diffusion can be hindered if the fluorophore binds to a larger molecule as shown in Figure 1a. In this study, we demonstrated a droplet-based microfluidic assay for protein-protein interactions in pico-liter droplets by fluorescence polarization using angiogenin (ANG) and anti-angiogenin antibody (anti-ANG Ab) [1]. Angiogenin is a 14-kDa small polypeptide implicated in angiogenesis and in tumor growth.

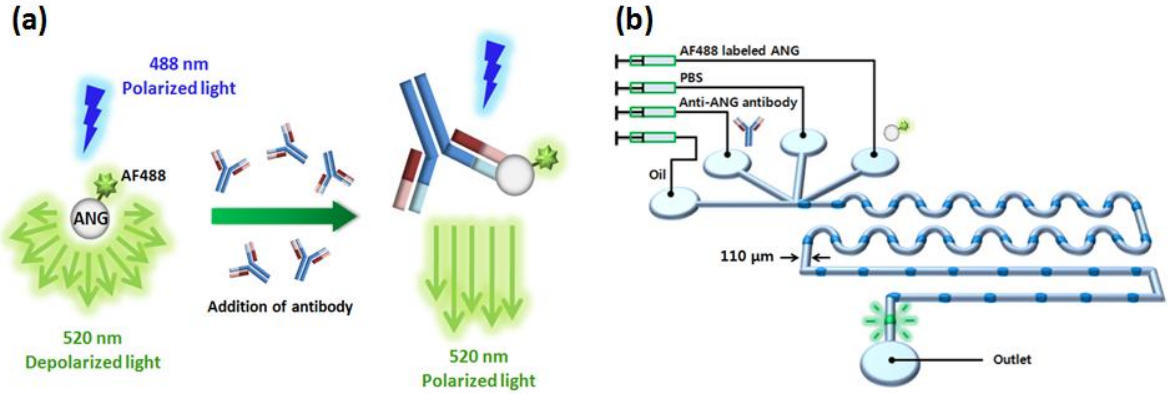

Figure 1. (a) Schematic of the fluorescence polarization facilitated by protein-protein interactions. (b) Layout of the microfluidic device for experiment. The AF 488 labeled angiogenin (ANG) solution is flowing into the right inlet, while anti-ANG antibody solution is flowing into the left inlet. The phosphate buffered saline (PBS, pH 7.4) is delivered into the middle inlet.

EXPERIMENT

We used angiogenin (ANG) and anti-angiogenin antibody (anti-ANG Ab) as an example of analysis of protein-protein interaction. Rosetta strain *E. coli* (carrying plasmid pET-angiogenin) were used for the expression of ANG. Anti-ANG Ab was isolated and purified from serum of rabbit. The purified ANG was labeled with Alexa Fluor 488 (AF488) dye, using AF488 protein-labeling kit according to manufacturer's protocol. Also we used 0.1 mg/mℓ bovine serum albumin in phosphate buffered saline (PBS, pH 7.4) for dilution.

Polydimethylsiloxane (PDMS) microfluidic devices were fabricated using standard soft lithographic techniques.

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As shown in Figure 1b, microfluidic devices consists of simple 3-inlet with T-junction. Three input aqueous phases were used in all experiments and consisted of angiogenin labeled with AF488 (AF488-ANG), anti-ANG Ab, and PBS as shown in Figure 1b. Also, 4% Abil EM 90 in mineral oil was used as a carrier fluid.

As shown in Figure 2, the fluorescence optical system consisted of a 488 nm diode laser, an inverted fluorescence microscope, and a dual polarization detection system. An electron multiplying-charge coupled device was used for the detection of polarization emission in experiments. Also bulk fluorescence polarization was measured on the Beacon 2000 Fluorescence Polarization System (Invitrogen) to compare with droplet-based microfluidic system.

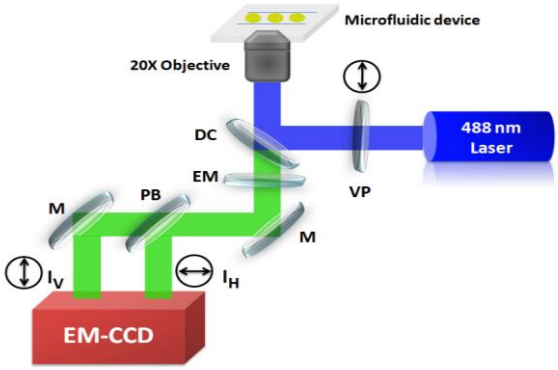

Figure 2. Schematic of the optical setup for fluorescence polarization mesurements. The system consists of 488 nm laser for fluorophore excitation, 20X objective, dichroic mirror (DC), emission filter (EM), vertical polarizer (VP), adjust mirror (M), polarizing beam-splitter (PB), and electron multiplying-charge coupled device (EM-CCD).

RESULTS AND DISCUSSION

To confirm the efficacy of our experimental approach, fluorescence intensity and polarization were measured as a function of AF488-ANG concentration in multiple droplets. Specifically, the concentration of AF488-ANG was varied between 0 and 10 nM by changing the relative flow rates of PBS steams from 0.1 to 0.9 μℓ/min, while the total aqueous flow rate was kept contant, maintaining both droplet size and generation frequency.

Figures 3a and 3b report the variation of fluorescence intensiy as a function of ANG concentration in both bulk and droplet environments. Over the concentration range studied, this variation is linear. Figures 3c and 3d describe the variation of fluorescence polarization over an identical concentration range. Not surprisingly, in both environments the polarization is of low magnitude and demonstrates no significant variation with AF488-ANG concentration.

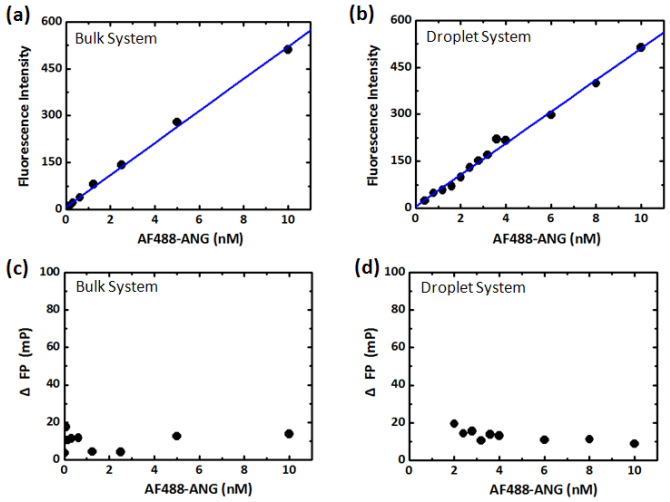

Figure 3. Fluorescence intensity (a, b) and polarization value (c, d) versus the concentration of the AF488-ANG in bulk fluorescence polarization system (a, c) and droplet-based microfluidic system (b, d).

Finally, the interaction between ANG and anti-ANG Ab was analyzed using fluorescence polarization within microdroplets. Solutions of AF488-ANG, PBS, and anti-ANG Ab were injected through the right inlet, middle inlet and left inlet, respectively. This PBS solution was used to separate AF488-ANG and anti-ANG Ab streams prior to droplet formation. Accordingly, on-line dilution was performed by varying the relative flow rates of anti-ANG Ab and PBS streams from 0.1 to 0.9 μℓ/min, but keeping AF488-ANG flow rate at 0.5 μℓ/min. The total aqueous flow rate was kept constant to maintain both droplet size and generation frequency as shown in Figure 4. Using these conditions, AF488-ANG was maintained at a concentration of 5 nM, while anti-ANG Ab was varied between 0.4 to

54 nM. Figure 5 demonstrates the variation in polarization as a function of anti-ANG Ab concentration. It can be seen that polarization increases with anti-ANG Ab concentration asymptotically approaching a maximum value at high anti-ANG Ab concentrations. The variation in polarization due to the interaction between AF488-ANG and anti-ANG Ab. A nonlinear least-squares fit to the data yields K_D = 10.4 ± 3.3 nM. For a comparison, K_D = 4.1 ± 0.9 nM extracted from bulk experiments (Figure 5a) and K_D = 16.6 ± 2.4 nM from previous droplet-based experiments using time-integrated fluorescence detection [2].

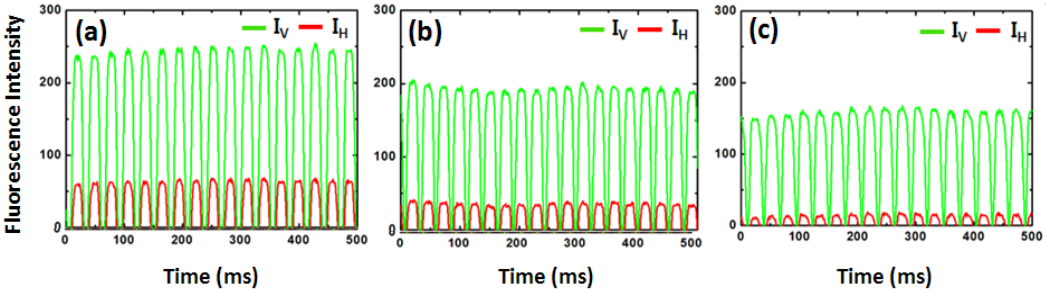

Figure 4. Examplar fluorescence burst scans recorded over a time period during 500 ms. The concentration of AF488-ANG was fixed at 5 nM, while the concentration of anti-ANG Ab was varied (a) 0.8 nM), (b) 12 nM, (c) 45 nM. I_V and I_H mean vertical intensity and horizontal intensity, respectively.

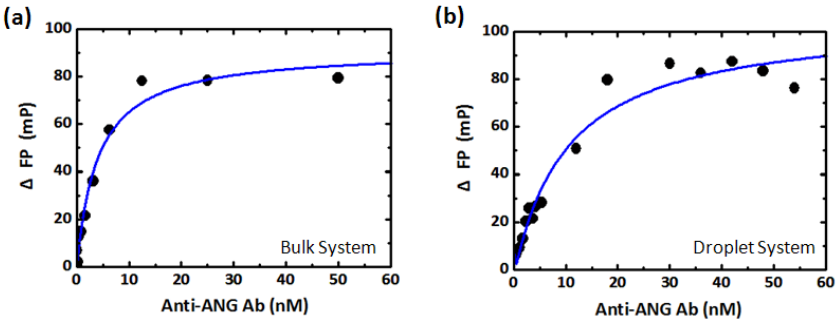

Figure 5. Analysis of interaction of angiogenin (ANG) with anti-angiogenin antibody (anti-ANG Ab) by fluorescence polarization. (a) In bulk system, the concentration of AF488-ANG was fixed at 1.25 nM, while the concentration of anti-ANG Ab varied 0 to 50 nM. (b) In droplet-based microfluidic system, the concentration of AF488-ANG was fixed at 5 nM, while the concentration of anti-ANG Ab varied 0 to 54 nM.

When operating at a droplet generation frequency of 40 Hz and an average droplet volume of 350 pℓ only 14 nℓ of sample is required per experiment. This represents a reduction of 4 orders of magnitude when compared to equivalent bulk assays.

CONCLUSION

Herein, we have demonstrated the combination of fluorescence polarization and droplet-based microfluidics for rapid analysis of protein-protein interactions. Specifically, the interaction between ANG and anti-ANG Ab was successfully analyzed in short times and high precision. A significant advantage when using fluorescence polarization to report protein-protein interactions is the requirement for only one labeled molecule. This contrasts with other approaches such FRET, which necessitate the labeling of both ligand and analyte. Importantly this means that the droplet-based fluorescence polarization assays is suitable for high-throughput screening and diagnostic measurements.

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