ANALYSIS OF ANGIOGENIN-ANTI-ANGIOGENIN ANTIBODY INTERACTIONS USING DROPLET-BASED MICROFLUIDICS

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ABSTRACT

The manipulation of multi-phase flows in microfluidic systems has been introduced as a fundamental experimental platform for high-throughput experimentation. In this study, we apply fluorescence resonance energy transfer (FRET) measurements in a segmented flow microfluidic platform to the analysis of protein-protein interactions. Angiogenin (ANG) is used as a model protein to confirm the efficacy of our experimental approach. Specifically, an anti-ANG antibody (anti-ANG Ab) and an ANG antigen are labelled with fluorophores to act as donor and acceptor in the FRET measurements.

KEYWORDS: Microdroplet, Protein-proetin interaction, Angiogenin, Angiogenin antibody

INTRODUCTION

The manipulation of multi-phase flows in microfluidic systems has been introduced as a fundamental experimental platform for high-throughput experimentation [1,2]. These systems enable the generation and manipulation of monodisperse bubbles or liquid droplets in an immiscible carrier fluid. Indeed, droplet-based microfluidic systems have been applied to a range of chemical and biological problems including enzymatic assays, protein crystallization, nanomaterial synthesis, high-throughput binding assays, real-time binding kinetics, and cell-based assays. Compared to conventional single-phase microfluidic systems, localization of reagents within discrete and isolated droplets has been shown to be an extremely effective way of enhancing reaction yields for diffusion limited reactions and eliminating residence time distributions. Moreover, the ability to controllably generate droplets with changeable reagent composition and at rates in excess of 1 kHz means, in theory, that millions of individual reactions or assays can be processed in very short times.

We apply fluorescence resonance energy transfer (FRET) measurements in a segmented flow microfluidic platform to the analysis of protein-protein interactions [3]. Angiogenin (ANG), a small polypeptide implicated in angiogenesis and in tumour growth, has a unique ribonucleolytic activity and undergoes nuclear translocalization in proliferating endothelial cells [4,5]. It is used as a model protein to confirm the efficacy of our experimental approach. Specifically, an anti-ANG

978-0-9798064-2-1/μTAS2009/$20

antibody (anti-ANG Ab) and an ANG antigen are labelled with fluorophores to act as donor and acceptor in the FRET measurements.

EXPERIMENTAL

The acceptor (Alexa Fluor 647; AF647) was linked with the donor (Alexa Fluor 488; AF488) by antigen-antibody binding (Figure 1a). Upon between anti-ANG Ab-AF488 and ANG-AF647 binding, the fluorophores are brought into close enough proximity for FRET to occur (Figures 1b**)**. Droplet-based binding experiments were performed using a polydimethylsiloxane (PDMS) microfluidic device containing three aqueous inlets, one oil inlet and a single outlet. A schematic of the microfluidic device is shown in Figure 1c. Here, anti-ANG Ab-AF488 is delivered via the left inlet, while ANG-AF647 is delivered through the right inlet. A central buffer stream is introduced through the middle inlet to prevent mixing of sample streams prior to droplet formation. This arrangement ensures that the binding and subsequent FRET occur only after the sample has been encapsulated inside a droplet. A two-colour fluorescence detection system (Figure 1d) was used to simultaneously record green and red fluorescence from the energy donor (AF488) and the energy acceptor (AF647) moieties, respectively.

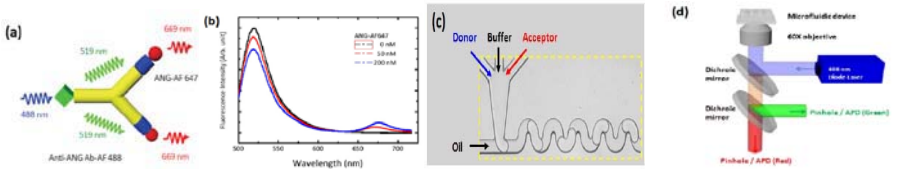

Figure 1. (a) Schematic of FRET process due to protein-protein binding. (b) Exemplar fluorescence emission spectrum. The anti-ANG Ab-AF488 concentration was fixed at 10 nM, whilst the ANG-AF647concentration was varied (0, 50 and 200 nM), (c) An example image of droplets generated with the indications of flow directions, and (d) Schematic of a built-in optical measurement setup.

RESULTS AND DISCUSSION

Typical FRET fluorescence burst scans over a time interval of 1 second are presented in Figure 2. Significantly, green and red signals, corresponding to AF488 and AF647 emission respectively, are coincident due to FRET.

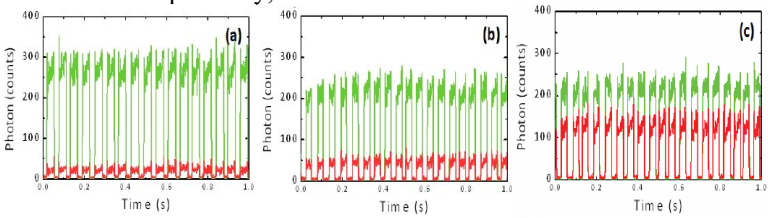

Figure 2. Exemplar fluorescence burst scans recorded over a time period of 1 second using a 50 s bin time. The anti-angiogenin antibody labelled with Alexa Fluor 488 (anti-ANG Ab-AF488) concentration was fixed at 10 nM, while the angiogenin labelled with Alexa Fluor 647 (ANG-AF647) concentration was varied from (a) 0.6 nM to (b) 3.0 nM and (c) 6.6 nM. The green signal represents anti-ANG Ab-AF488 fluorescence and the red signal represents ANG-AF647 fluorescence.

Measurement of the K_D values of ANG and anti-ANG Ab from the droplet-based microfluidic experiments (K_D = 16.6 ± 2.4 nM) is shown to agree closely with data obtained from bulk fluorescence polarization measurements (9.0 ± 1.5 nM) (Figure 3). These results suggest that droplet-based measurements reach equilibrium prior to the acquisition of fluorescence data.

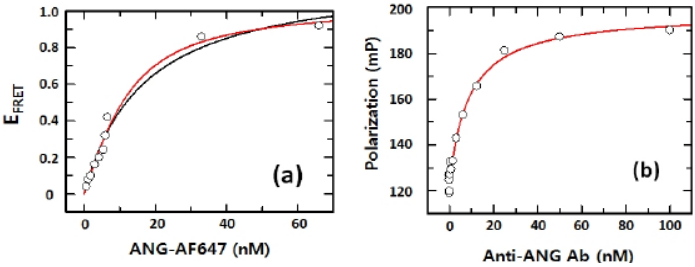

Figure 3. Binding of angiogenin (ANG) with anti-angiogenin antibody (anti-ANG Ab) by (a) droplet-based microfluidic experiments measurements (black line, non-tight binding fit; red line, tight-binding fit) and (b) bulk fluorescence polarization.

CONCLUSIONS

We have successfully demonstrated FRET-based analysis of protein-protein interactions in thousands of picoliter-sized droplets. We expect that such an experimental platform will have significant applicability in the high-throughput analysis of protein-protein interactions.

ACKNOWLEDGEMENTS

This work was supported by the research grant of the Chungbuk National University in 2007.

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