HIGH THROUGHPUT DROPLET-BASED DNA ASSAYS USING FLUORESCENCE RESONANCE ENERGY TRANSFER

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ABSTRACT

Herein, we present a droplet-based microfluidic device integrated with a confocal fluorescence detection system which is used to perform high-throughput binding DNA assays relying on fluorescence resonance energy transfer (FRET). The exploitation of confocal detection allows for online characterisation of individual droplets and their contents in terms of their size, formation frequency, fluorescence intensity and population.

Keywords: droplets, high throughput analysis, binding assay, FRET

1. INTRODUCTION

Droplet-based microfluidic systems have been utilised to address a variety of problems in the chemical and biological sciences [1]. Droplets can be controllably produced at rates in excess of 1KHz onwards with each droplet acting as an isolated reactor. In theory, this means that tens of thousands of individual reactions or assays can be processed in very short time scales. Accordingly, highly efficient detection plays a crucial role in extracting and utilising the vast amounts of information produced in such systems. To this end, we report herein, a confocal fluorescence detection system integrated with droplet-based microfluidic devices to perform high-throughput DNA binding assays based on FRET. Through the integration of high-sensitivity confocal spectroscopy, we demonstrate that high-speed picoliotre-sized droplets in continuous flow can be characterised online and analyte inside such droplets can be accurately and precisely quantified. The virtue of droplet systems in terms of compartmentalisation, negligible reagent dispersion and rapid mixing via chaotic advection is advantageous to laminar flow for studying biological screening and assays.

2. EXPERIMENTAL

Detection setup: A custom built confocal fluorescence detection system for droplet detection is schematically shown in Figure 1a. **Binding assays:** PDMS microfluidic devices, having 50 μm wide, 50 μm deep and ~44 cm long channels, were used. By attaching a FRET donor (Alexa Fluor 488) to streptavidin and labelling a FRET acceptor (Alexa Fluor 647) onto one DNA strand hybridised to a biotylinated complimentary strand, donor and acceptor molecules were brought into close proximity due to streptavidin-biotin binding, resulting in FRET (Figure 1b). To create a microdroplet system, donor-labelled streptavidin and acceptor-labelled hybridised DNA solutions were introduced separately through the aqueous inlets of a microfluidic device to meet a stream of an immiscible oil (continuous) phase, a 10:1 (v/v) mixture of perfluorodecalin and 1*H*,1*H*,2*H*,2*H*-perfluorooctanol, at a T-junction. The concentration of streptavidin was fixed at a desired value whereas the concentration of DNA was ramped by continuous and precise control of input flow rates to assess binding. The flow rates of the oil phase and aqueous solutions

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were kept constant at 1.5 µl min^{-1}, resulting in a total flow rate of 3.0 µl min^{-1} (20 mm s^{-1}). A schematic of the microfluidic device topology and a sample image of formed droplets are illustrated in Figures 1c and 1d. A buffer stream was delivered into the middle aqueous inlet to prevent reagents from coming into contact prior to droplet formation and to allow for online dilution. Thus, mixing and binding of streptavidin and DNA only occurred inside the droplets. Online fluorescence detection was conducted at ~42 cm or ~20 s (the total channel length is ~44 cm) downstream from the droplet-forming region. Two fluorescence signals, from the donor and acceptor, were recorded simultaneously using separate avalanche photodiode detectors (APDs).

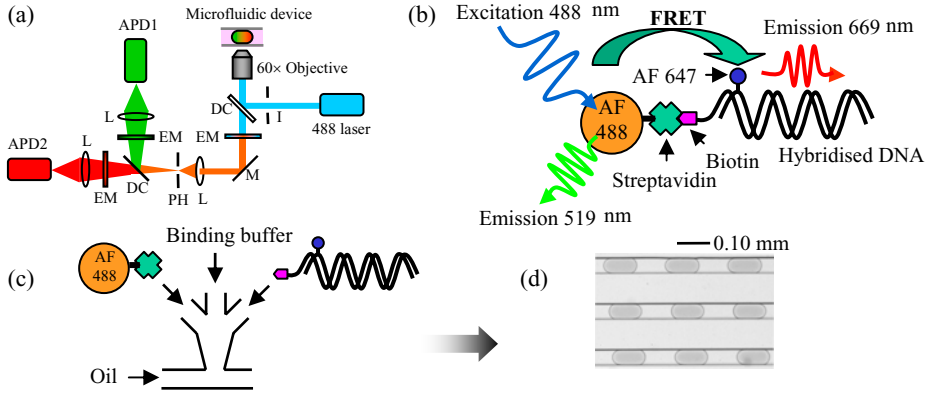

Figure 1. (a) A home built confocal microscope used for droplet detection: APD-avalanche photodiode detector, DC-dichroic mirror, EM–emission filter, I-iris, L-lens, M-mirror and PH-pinhole. (b) FRET process using Alexa Fluor 488 and Alexa Fluor 647 as a donor and an acceptor, respectively. (c) Schematic of microfluidic device used for FRET biological assays. (d) Example image of droplet generated using the microfluidic system.

Binding kinetics: Streptavidin-biotin binding kinetics were also investigated via FRET. Streptavidin and DNA concentrations were fixed at 22.0 and 60.0 nM, respectively, resulting in a binding ratio of 2.73, defined as the ratio between DNA and streptavidin concentrations. Droplet detection was performed by probing the microfluidic channel at different points to extract the binding kinetics.

3. RESULTS AND DISCUSSION

FRET binding assays: An example of a FRET burst scan from the confocal system is shown in Figure 2a. Importantly, the donor and acceptor signals were coincident and only ~1.2% crosstalk (leakage of donor emission into the acceptor channel) of this FRET pair was observed. This indicates that the majority of the signal associated with the acceptor is due to FRET. A plot of binding ratio as a function of the 'red' burst height and fit using the Hill-Waud model [2] are depicted in Figure 2b. Red fluorescence bursts increased with the binding ratio due to a result of greater energy transfer with a higher number of acceptors linked to the donor. Comparison of data measured at different streptavidin concentrations demonstrates that the higher the streptavidin concentration, the higher the energy transfer (due to the increased number of donors to transfer energy). It was observed that all binding curves plateau at binding ratios of ~2.0, indicating that only 2 biotin binding sites on streptavidin were successfully filled.

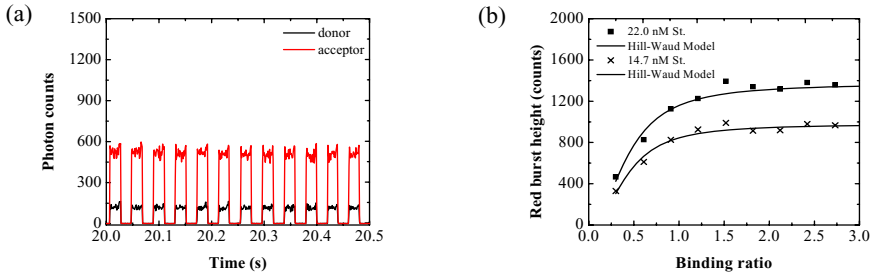

Figure 2. (a) A 500 μs fluorescence burst scan. Data was recorded using a 50 μs resolution. (b) Binding curves.

Figure 3a shows plots of FRET efficiency calculated from the ratio of acceptor fluorescence intensity to the summation of acceptor and donor fluorescence intensities. It can be seen that the FRET efficiency of this system is ~0.75.

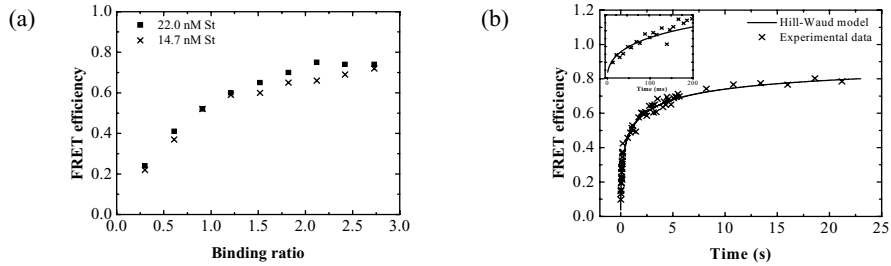

Figure 3. (a) Plots of FRET efficiency as a function of binding ratio. (b) Streptavidin-biotin binding kinetic plot.

FRET binding kinetics: Figure 3b illustrates a streptavidin-biotin binding map plotted using FRET efficiency against time. An inset over 200 ms shows high resolution kinetic measurements on a millisecond timescale. The FRET efficiency drastically increases at the beginning of the curve and remains stable after ~5.0 s. This implies that complete binding of our analytes occurs in a 5 s time frame.

4. CONCLUSIONS

Droplet-based microfluidics integrated with confocal microscopy was successfully exploited to perform high-throughput streptavidin-biotin binding assays. The binding ratio between streptavidin and biotin was found to be ~2.0 with the binding kinetics of ~5.0 s. High FRET efficiency of ~0.75 was obtained from this system. Using this new approach, high-throughput analysis or screening is a highly promising direction for future research.

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