DYNAMIC IMAGING OF
SINGLE BIOMOLECULAR INTERACTION
USING FLOW CONTROL AND TIRFM
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ABSTRACT
A rapid multi-reagents switching microvalve system integrated with a total internal reflection fluorescence microscopy (TIRFM) was developed for real time imaging of a single protein behavior. The binding and dissociation process between a chaperonin GroEL and cochaperonin GroES was observed in this TIRFM microfluidic system. For single molecular imaging in this system, biotinylated GroEL (D490C) was immobilized to the glass microchannel surface through streptavidin and biotinylated BSA. A solution including 1 nM IC5-GroES was introduced for 100 ms into the observation area and then washed out with 2 mM ATP buffer solution. Fluorescence spots of IC5-GroES appeared after rapid solution switching, and disappeared several seconds later. As a result, we succeeded in detecting fluorescence signal from single molecules in TIRFM microfluidic system.

KEYWORDS: TIRFM, Single biomolecular imaging, Microfluidic device

INTRODUCTION
Single molecular detection and sensing of biomolecules are one of the next trends in LOC. Single molecule imaging by TIRFM is most promising in this field. The TIRFM technique is applied for single biomolecular observation under low background noise using evanescent field, which is produced beyond the interface with penetration depth of 150 nm when a laser is incident on the quartz substrate above the total reflection angle \[1\]. The low background fluorescence light allows video-rate imaging of single fluorescent molecules. This real-time single molecule imaging technique in aqueous solution has many advantages for analysis of single molecule movement in vitro, behavior of protein on the surface of living cells.

In this paper, we describe an in-channel PDMS-glass microvalve and its application for TIRFM, which is promised single molecular level imaging, detection and sensing of biomolecules. The experimental evaluation of the fabricated microfluidic system was performed by single molecule imaging of chaperonin GroEL–GroES interaction under rapid multi-reagent switching and TIRFM observation.

EXPERIMENTAL
Developed TIRFM microfluidic system contains three inlet channels and one outlet channel and the flow in every inlet channel were precisely controlled by the PDMS microvalves (Figure 1). This system was constructed by a smooth glass in
micro-channel surface, that is essential for TIR imaging, and the PDMS microvalves that is operable with a rapid response of around 100 ms. The smooth glass surface was etched with an optimized hydrofluoric acid and nitric acid (HF-HNO₃) solution and had the average surface roughness of 3.2 nm.

RESULTS AND DISCUSSION

To estimate the viability of single molecule imaging using this system, we observed dissociation of GroES from GroEL. Schematic drawing of experimental procedure is shown in Fig.2. First, biotinylated GroEL was immobilized to the glass surface through streptavidin and biotinylated BSA as described [2] (Fig.2a). 1 nM IC5-GroES was introduced for 100 ms into the imaging area and then washed out with buffer. In the absence of ATP, few spots of IC5-GroES appeared after solution switching experiment. In the presence of 2 mM ATP, fluorescence spots of IC5-GroES appeared after solution switching, and disappeared several seconds later (Fig.2b,c). This result indicated that fluorescence signal detection from single molecules was performed in TIRFM microfluidic system. Furthermore, the appearance of these spots depended on ATP, indicating that almost all the spots were IC5-GroES attached to GroEL specifically and not IC5-GroES adsorbed to glass surface nonspecifically. Next, we have analyzed the time course of fluorescence intensity of IC5-GroES. Fluorescence signal was almost constant for several seconds and disappeared with single steps (Fig.3a,b). Histogram of the duration of GroES binding was fit by the following equation (1) using k = 0.60 s⁻¹ and k’ = 0.35 s⁻¹ (n=169) (Fig.3c).

Fluorescence intensity = C [k’ exp(-kt) – k exp(-k’t)] / (k’ – k)                     (1)
where $k$ and $k'$ are rate constants. $C$ represents a constant value and $t$ is the duration of binding of GroES and GroEL. The effect of photobleaching was negligible because photobleaching rate of IC5 was $0.021 \text{s}^{-1}$ in this experimental condition.

**CONCLUSIONS**

Multi-reagents switching microfluidic system integrated with a TIRFM was fabricated and performed. The automatic flow control system was established for real time monitoring of chaperonin GroEL-GroES interaction at single molecular observation. It will be applicable to kinetic study of various kinds of biomolecules and will broaden the potentiality of single molecule imaging under quantitatively controlled multi-reagent exchange in TIRFM microfluidic system.

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