MICROFLUIDIC IMMOBILIZATION AND PROGRAMMABLE RELEASE OF SINGLE MOLECULES

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Abstract

We report a novel approach that permits isolation, immobilization and controlled release of individual DNA molecules within microfluidic channels, for use in single molecule analysis systems. Single molecule DNA analysis systems have made substantial progress during the past decade [1]. However, one of the obstacles that limit the use of single molecule analysis technology is the lack of an efficient, high throughput method for isolating, immobilizing, and manipulating individual molecules. We have experimentally demonstrated a multi-step process that consists of isolation and immobilization of individual DNA molecules within a microfluidic channel, followed by controlled release of individual molecules into solution by electrical heating.

Keywords: **single molecule, immobilization, DNA, microfluidics, release**

1. Introduction

Single molecule research has gained considerable attention during the past decade [2]. This has been largely due to the increasing interest in understanding the behavior and properties of bio-molecules and in particular, nucleic acids (DNA and RNA). Tremendous advances have been in both isolation and manipulation of individual molecules, particularly DNA. Generally, single molecule isolation and manipulation is achieved by modifying the ends of DNA molecules with various functional groups such that they covalently bind to complementary groups attached to solid supports such as beads or surfaces. Beads have been used as handles for manipulating DNA using various techniques such as optical tweezers [3]. The major drawback in these techniques is the difficulty in limiting the number of molecules that become attached to the beads or surfaces. The challenge therefore lies in reliably and efficiently isolating or attaching one DNA molecule per bead. We address this problem by combining DNA hybridization chemistry with microfluidics to spatially isolate single molecules at multiple locations within a microfluidic channel. We further demonstrate that heating of the isolated, hybridized molecules can effectively act as a single molecule dispenser.

The strategy for single molecule isolation and release within microfluidic channels is shown in Figure 1. Single molecule isolation was achieved by immobilizing 'anchor' DNA on the surface of a microfluidic channel and using it to hybridize to 'target' DNA molecules. Single molecule release of the 'target' DNA molecules is then achieved on demand by heating the microchannel using a thin-film resistive heater. We have demonstrated this approach using a PDMS-based microfluidic chip. Both static (no flow) and dynamic experiments (within microfluidic channels) for

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single molecule isolation and immobilization were performed. Single molecule isolation and dispensing were experimentally demonstrated, and visualized through the use of streptavidin-coated beads.

2. Experimental

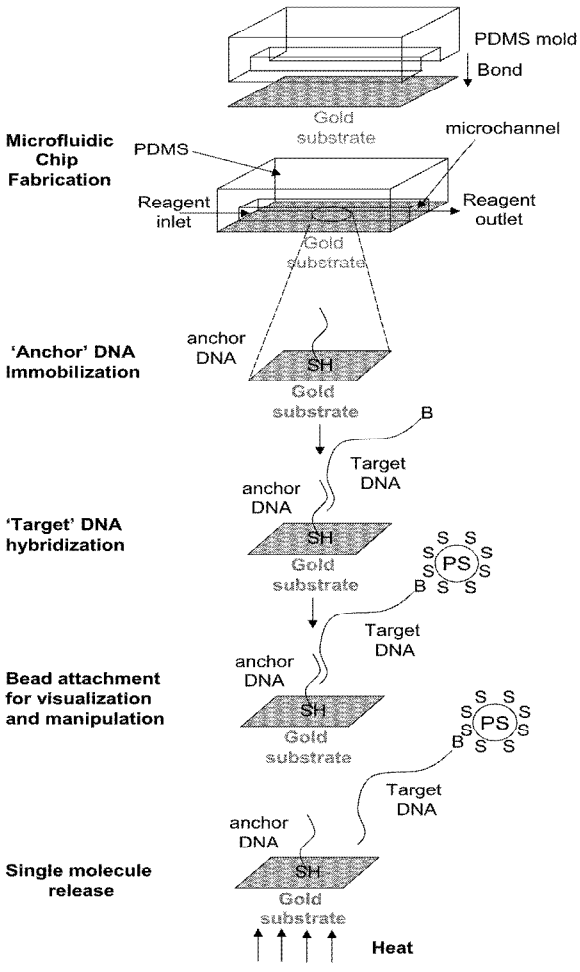

Figure 1 – Strategy and procedure for single molecule isolation, immobilization and dispensing within microfluidic channels.
Key: SH – Thiol; B – Biotin; S – Streptavidin; PS – Polystyrene bead (1 μm dia.).

Substrate Fabrication: Gold substrates used for attachment chemistry were prepared by sputter deposition of 80 Å of Chromium followed by deposition of 240 Å of gold.

Microfluidic Chip fabrication: Designs of the micro fluidic channels to be fabricated were drawn to scale using CAD software. The designs were then printed onto transparencies using a high-resolution printer. The channels were ~ 100 μm in width and 2-3 cm in length. "Photoresist on Silicon" masters for micromolding were prepared by standard photolithography using the transparency masks and SU-8 photoresist. These patterned masters were then silanized and used for micromolding with poly (dimethyl siloxane) (PDMS). PDMS precursor was poured onto the silanized master and then cured. The cured PDMS containing the channel structure was then bonded to the gold substrate by applying pressure to enclose the channels.

'Target' DNA:
Modified λ-phage DNA (48.5 kbps) was used as the target DNA in this study. λ-phage DNA was modified through ligation using DNA oligos such that one end has a complementary sequence that hybridizes to the 'anchor' DNA, and the other end has a biotin label for attachment to the polystyrene (PS) bead. Modified λ-DNA molecules after ligation were separated from the short DNA oligos by polyethylene glycol precipitation method. Precipitated 'target' DNA was collected and dissolved in buffer and stored at 4 deg. C before use.

The following procedure was used for the static (no flow) experiments:

Immobilization of 'anchor' DNA on gold substrate: Thiol-modified 'anchor' DNA oligo was pipetted onto the surface of the gold thin film substrate and incubated at room temperature for 3-4 hrs. The surface was then washed with 1xPBS several times to remove unbound oligos.

Immobilization of target DNA via hybridization: 'Target' DNA was pipetted onto the substrate and incubated at room temperature for 1-2 hrs. After hybridization, the substrate was then rinsed with 1xPBS (>3x) to remove unhybridized DNA molecules.

Bead attachment: After hybridization, a solution of streptavidin-coated polystyrene (PS) beads (1 μm dia.) was incubated on the substrate for 1 hr to attach the beads to the biotinylated end of the 'target' DNA for visualization and to serve as handles for optical manipulation after release.

For the dynamic (within microfluidic channels) experiments:

The reagents were pumped through the channels in the same order as in the static case, for 5 min by applying vacuum, followed by incubation within the microfluidic channels for time periods comparable to those used in the static experiments.

3. Results and Discussion

Single molecule isolation in static conditions: The density of single 'target' DNA molecules hybridized using static conditions was 3-5 molecules per 100 μm x 100 μm square area. The beads attached to 'target' DNA exhibited Brownian motion but were restrained to within a radius of the < 2-3 μm. The DNA immobilization and the bead attachment were further confirmed by using a standard upright optical microscope equipped with optical tweezers by trapping and pulling the beads attached to the 'target' DNA molecules.

Single molecule isolation within microfluidic channels: Microfluidics permitted easy identification of which DNA/bead complexes are immobilized. It was noticed that the efficiency of hybridization within microfluidic flows was lower than in the static case as expected, allowing single molecule isolation to be achieved at multiple dispersed locations. The number of single molecules isolated within a microfluidic channel of 100 μm width and 1 cm length was approximately 10-20 molecules.

Single molecule release within microfluidic channels by electrical heating: After visualization of single molecule immobilization, single molecule release is then achieved by heating the chip. This is performed using a thin film resistive heater located underneath the chip and controlling the current passing through the resistive heater. When the local temperature at the anchor point exceeds the melting

temperature of the hybridized DNA molecule, it denatures and gets released from the anchor DNA (Figure 2). For the DNA sequences that were chosen, the theoretical release temperature was 48.9 deg. C. The releases of various single DNA molecules isolated were observed at substrate temperatures ranging from 46 deg. C to 53 deg. C. This range was observed for single molecules released from the same microfluidic channel, as well as from multiple channels.

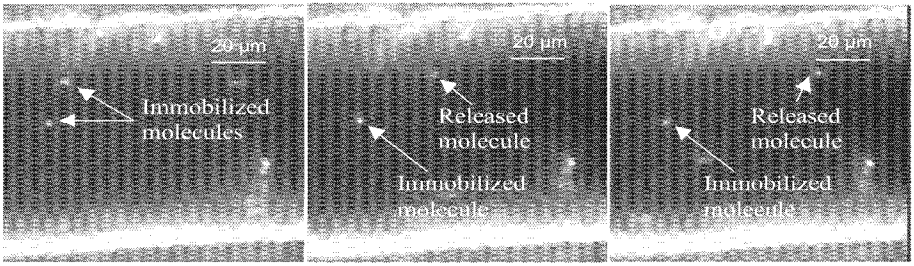

Figure 2 - Sequential frames from video microscopy of single molecule release (Left) Immobilized DNA molecules with 1 μm poly-styrene (PS) beads (Center) Release of a single DNA molecule into the flow upon temperature increase (Right) Released DNA molecule flows to the right while the immobilized molecule remains anchored. Other beads shown in these frames do not have DNA attached, and were used to illustrate fluid

4. Conclusion

We have demonstrated a novel approach that permits isolation, immobilization and controlled release of individual DNA molecules within microfluidic channels. This approach makes it practical to isolate and selectively dispense individual molecules in microfluidic single molecule analysis systems. Future work could include careful design of the 'anchor' DNA sequences to permit control of temperature at which the DNA molecules get released, use of localized heating methods, and statistical characterization of the single molecule isolation density.

References

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