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COUPLED FREE-SOLUTION SEPARATION AND ON-CHIP HYBRIDIZATION OF OLIGONUCLEOTIDES IN A NANOFLUIDIC DEVICE

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ABSTRACT

While the challenges and advantages of micro-scale analysis systems are well known, those of systems incorporating nano-scale features have only recently begun to be recognized. One such advantage is the capability to perform novel separations based upon nano-scale physical phenomena [1]. In this work, we present experimental results demonstrating on-chip oligonucleotide hybridization and free-solution (gel-free) separation of hybridized (ds) DNA from single-stranded (ss) DNA using a fundamentally new separation mechanism inherent to nanofluidic channels.

Keywords: nanofluidics, separations, hybridization, oligonucleotide

1. INTRODUCTION

The most common hybridization assays are DNA microarrays, which use surface-bound probes and require large amount of DNA and long hybridization times. The most common means of separating DNA, for detection and sequencing, is through the use of a sieving matrix. The addition of sieving matrices, however, makes it difficult to interface with upstream hybridization assays or additional downstream manipulations or studies. At Sandia, we are working to develop a coupled free-solution separation and hybridization assay for applications in rapid biodetection and bioanalysis.

2. THEORY

By performing free-solution hybridization assays in nanochannels, we take advantage of both the improved mixing times afforded by the reduced channel size [2] and the separation capabilities of nanochannels, initially demonstrated by Pennathur and Santiago [3]. In a nanochannel, the electrical double layer occupies a significant proportion of the fluid volume, generating transverse electric fields that couple with nonuniform velocity fields to cause a valence-based separation modality [1]. In addition, larger molecules such as DNA also experience steric interactions with the wall, which vary with the length of the DNA and also contribute to the separation mechanism [4]. We take advantage of the conformational difference between single-stranded and double-stranded DNA (e.g., the relative stiffness and persistent lengths) to perform separations in a nanochannel. Here, we present the on-chip hybridization of fluorescently-labeled oligonucleotides and separation of a 20-mer double-stranded DNA from its unhybridized constituents.

3. EXPERIMENTAL

Hybridization and separation were performed in fused-silica nanochannels fabricated using conventional MEMS processing techniques [3]. As depicted in Figure 1, the device consisted of a 5-port structure. Ports A and B form a mixing Tee which feeds into a standard offset-T injection structure, with the separation channel extending from the injection region to the buffer waste port. The nominal channel depth is 400 nm and the width is 10 μm. Full-field fluorescence images were captured using a CCD camera (Cascade 128+, Roper Scientific) mounted on an epifluorescent microscope (Nikon TE-2000). Electropherograms were generated by averaging a 100 pixel section of the channel at a distance 22 mm from the injection region. Figures 2a and 2b demonstrate the successful separation of hybridized probe and target DNA from unhybridized probe. Figures 3a and 3b presents electropherograms for on-chip hybridization at two different hybridization times.

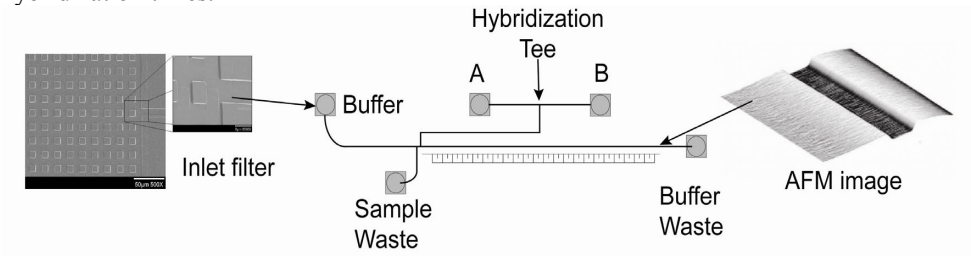

Figure 1. Device schematic and detail images. The schematic illustrates the 5-port device layout, including sample wells (A and B), mixing Tee, offset Tee injector, and separation channel (leading to the buffer waste port and indicated by the tick marks beneath). The left-most image is a scanning electron micrograph of the port region, showing the filter blocks, while the right image is a representative AFM measurement of a channel section.

4. RESULTS AND DISCUSSION

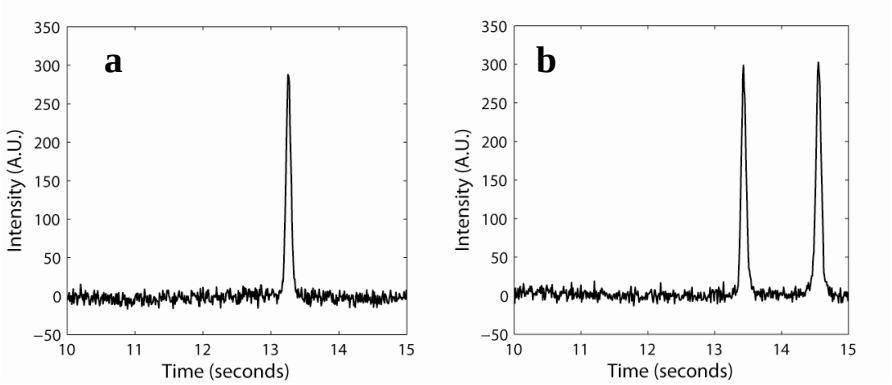

Figure 2a: Electropherogram of single-stranded DNA. The plot shows the resultant peak for a 20 base pair probe oligonucleotide labeled with Alexa Fluor 546. The single stranded DNA was loaded into port A at a concentration of 1 μM in a 10 mM borate buffer. The field strength in the separation channel was 360 V/cm. Figure 2b: Separation of single-stranded and double stranded DNA. The electropherogram shows the separation of

hybridized and unhybridized probe DNA. The separation conditions were identical to figure 2a, with the addition of 1 μM probe and 0.5 μM unlabeled complement in port B.

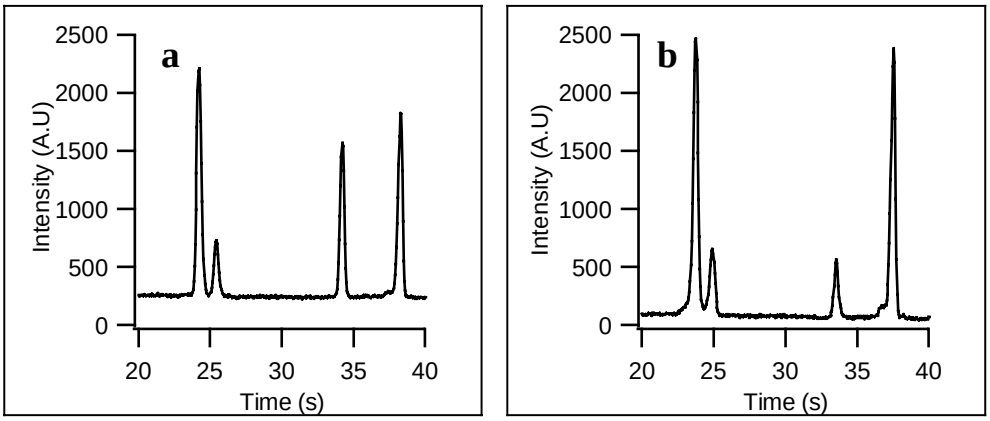

Figure 3a: On-chip hybridization studies. In the electropherograms above, the first pair of peaks are the reference dye markers (Fluorescein and Oregon Green) added to the single strand DNA solutions at Ports A and B, respectively. The second pair of separated peaks show the relative concentration of the unhybridized and hybridized DNA with no additional mixing or hybridization time. Whereas in Figure 3b, the applied field is turned off for 300 s to increase hybridization time in the mixing Tee just prior to injection. A decrease in peak height is observed for the unhybridized ss-DNA peak and an increase in height for the hybridized ds-DNA peak with the longer mixing time, suggesting an improvement in hybridization. The separation conditions for the electropherograms are identical: 10mM Tris, 1mM EDTA background buffer with 1mM NaCl and an applied field strength of 720 V/cm.

5. CONCLUSIONS

We have demonstrated successful on-chip hybridization and oligonucleotide separation with a nanofluidic device using a fundamentally new separation mechanism inherent to nanofluidic channels.

ACKNOWLEDGEMENTS

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