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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 separation mechanism inherent to nanofluidic channels.

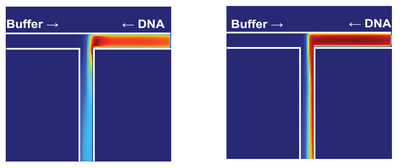
Background

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.

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.

[1] S. Pennathur and J.G. Santiago, Electrokinetic transport in nanochannels. 1. Theory, Anal. Chem., 77, pp. 6772-6781, (2005).
[2] S. Pennathur et al, Diffusion-based Free Solution DNA Hybridization and Transport in a Nanofluidic Device, Proc. Micro Total Analysis Systems 2006, Tokyo Japan, pp. 1079-1081, (2006).
[3] S. Pennathur and J.G. Santiago, Electrokinetic transport in nanochannels. 2. Experiments, Anal. Chem., 77, pp. 6782-6789, (2005).
[4] S. Pennathur et al, Free-solution oligonucleotide separation in nanometer scale fluidic channels, submitted to Lab on a Chip, (2007).

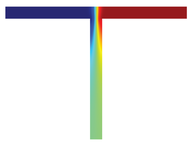
Mixing and Hybridization



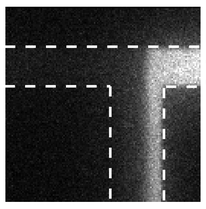
Field strength = 220 V/cm

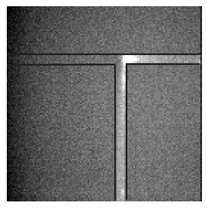
Field strength = 1340 V/cm

Mixing studies: fluorescent intensity images were captured at the mixing T. Buffer (10mM Tris, 1mM EDTA) and buffer spiked with with 5 μM DNA (20-mer oligonucleotides end-labeled with Alexa Fluor 546) were driven via electroosmosis through a mixing T junction. Images were averaged from 60 darkfield corrected frames and normalized against the maximum intensity.



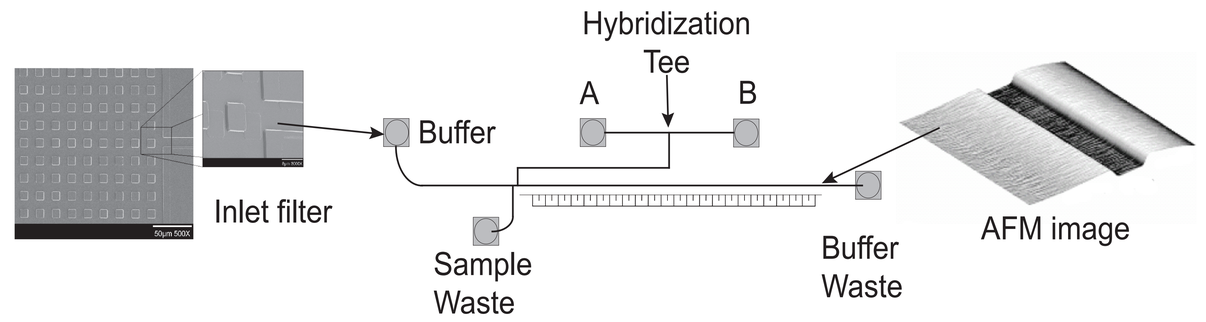
Simulated DNA concentrations in the mixing T as a function of electric field. The electrokinetic transport of oligonucleotides was modeled in COMSOL Multiphysics. The electric fields correspond to the experimental results above. The electrophoretic mobility and diffusivity were - 3 x 10^{-8} m^2/Vs and 9.7 x 10^{-11} m^2/s, and the electroosmotic mobility was 4 x 10^{-8} m^2/Vs.





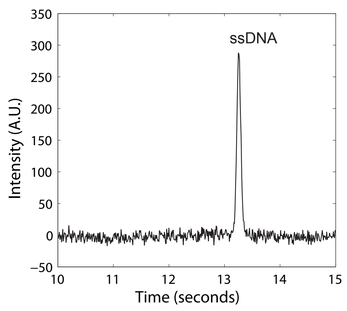
Hybridization studies: sample fluorescence intensity images are presented from on-chip hybridization studies. The left hand image depicts the interface between unlabeled oligonucleotide and a mixture of its complementary DNA and picogreen dye. Picogreen dye acts as a hybridization indicator, since its fluorescent intensity increases significantly in the presence of double-stranded DNA. The right hand image depicts the interface (at lower magnification) between an unlabeled target oligonucleotide and a complementary molecular beacon acting as a probe. Note the increase in fluorescence from the beacons at the T-junction.

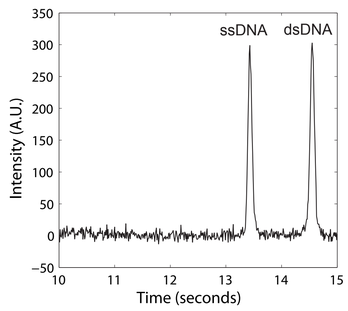
Nanofluidic Device



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. The device was fabricated with a range of depths (200nm to 800nm) in fused-silica using conventional photolithography and plasma etching [3].

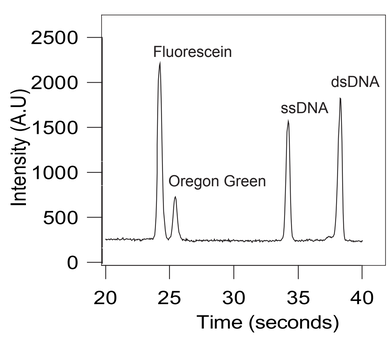
DNA Separation

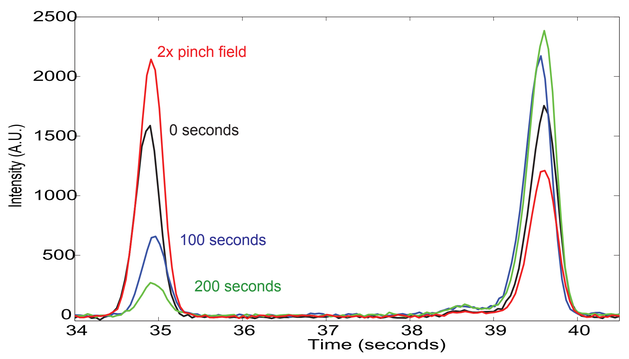




Separation of single- and double-stranded DNA: the left-hand electropherogram shows the resultant peak for a 20 base pair probe oligonucleotide labeled with Alexa Fluor 546. The single stranded DNA was loaded into port A at a concentration of 1 μM and the background buffer was 10 mM borate. The field strength in the separation channel was 360 V/cm. The right-hand electropherogram shows the separation of hybridized and unhybridized probe DNA. The separation conditions were identical, with the addition of 1μM of probe hybridized to its complement in port B.

Coupled Hybridization and Separation





Separations with on-chip hybridization: coupled on-chip hybridization and electrophoretic separation was performed in 10 mM Tris-EDTA buffer with 1 mM sodum chloride. A 20 base pair probe labeled with Alexa Fluor 488 was loaded in port A and unlabeled complement in port B, each at a concentration of 33 μM. Fluorescein and Oregon Green dyes were added as electrophoretic markers. The left-hand electropherogram shows a single separation at a field strength of 360 V/cm. To assess the impact of hybridization time, separations were performed varying the hybridization wait time from 0 to 200 seconds, as well as doubling the pinch field strength (reducing the hybridzation time in half). The right-hand plot shows the relative increase in double-stranded DNA as the hybridization time increases.

Acknowledgments

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