**Surface Diffusion of DNA Oligonucleotides on Patterned Silane Surfaces**
*Travis Crites and James W. Schneider*

In detection modalities that require waveguides, the study of lipid bilayers, and binding events on the surfaces of living cells, detection must often occur on flat surfaces. Here, dilute analyte concentrations often lead to large capture times owing to the long distances analytes must travel to reach probes and the fairly low probability of capture once they reach the probe. To speed the detection of analytes in planar biosensor, in our case DNA oligonucleotides (ssDNA), we are pursuing the use of patterned surfaces that direct their analytes to their complementary probes by surface diffusion. This reduction of dimensionality (RD) enhancement reduces the distance a molecule must traverse from bulk solution to reach a fixed detection site.

We have designed surfaces that possess domains to which ssDNA does not readily adsorb, surrounded by regions that encourage surface diffusion along the detection surface while allowing the analyte to sample many conformations resulting in higher capture probabilities. Surfaces were constructed from patterned self-assembled monolayers (SAMs) of the neutral octadecyltrichlorosilane (OTS). Following adsorption of OTS, a photomask with circular transparent patches was placed over the OTS surface and UV irradiated, leaving behind regularly patterned circles of glass surrounded by OTS. The remaining areas of bare glass are then backfilled with either aminohexylaminopropyltrimethoxysilane (AHTMS), with a positive amine terminus, 11-bromoundecyltrichlorosilane (BrUTS), possessing a bromine terminus, or methoxy(polyethyleneoxy)propyltrimethoxysilane (PEGS), a silane with 6-9 PEG monomer units at its terminus.

For surface diffusion to occur over reasonable time scales, the adsorbed DNA must be reversibly adsorbed to the pathways. This was assessed using a total internal reflection-fluorescence (TIRF) / fluorescence recovery after patterned photobleaching (FRAPP) apparatus on an inverted microscope stage. Using the TIRF/FRAPP instrument, we have studied adsorption, desorption, and surface diffusion of fluorescently labeled DNA oligonucleotides on patterned AHTMS, BrUTS, and PEGS SAMs. The use of TIRF ensures that only molecular processes occurring very close to the surface are detected. Total adsorbed amounts are measured by the TIRF intensity, while the FRAPP yields surface diffusion coefficients.

We tested the reversibility of adsorption on an AHTMS surface at different ionic strengths.

At all ionic strengths, an initial, fast adsorption was observed followed by a second, slower adsorption proceeding after 100 seconds. This can be explained by two sequential molecular processes, the first driven by electrostatic interactions, and the second driven by hydrophobic interactions or conformational rearrangement. Desorption experiments showed little desorption after a surface pre-adsorbed with DNA in pure buffer was rinsed with DNA-free buffer (50 mM

Tris HCl, pH 8.0) (**Figure 1**). However, nearly all of the material on the surface desorbed in low and high ionic strength buffers. This non-monotonic behavior again indicates that two distinct processes are controlling the adsorption. Further, the total adsorption at low and high ionic strengths was higher than in 50 mM Tris HCl. The good agreement of adsorption and desorption data demonstrate that the adsorbed DNA is in equilibrium with the DNA in solution as required for appropriately fast surface diffusion. Accordingly, surface diffusion coefficients measured by TIRF/FRAPP agree well with literature values on the AHTMS surface ($D_{surf} \approx 1 \times 10^{-8}$ $cm^2/s$).

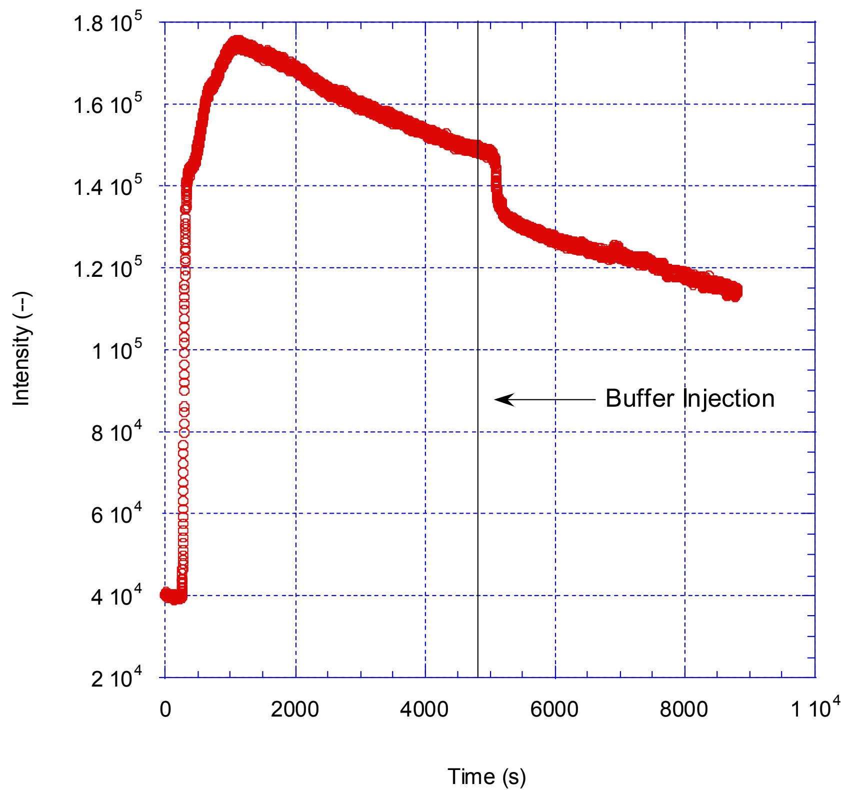


**Figure 1: Adsorption of ssDNA to AHTMS (50 mM Tris HCl, pH=8.0)**

Each of the three silane SAMs adsorbs ssDNA with a different affinity. After equilibration of the SAMs with hexachlorofluorescein(HEX)-labeled, 20-mer ssDNA (50mM Tris pH=8.0), they were imaged using an epi-fluorescent microscope. On AHTMS surfaces, no distinct domains can be seen. This is the expected result since the density of adsorbed ssDNA has been shown to be of the same order of magnitude on OTS and AHTMS. In the case of BrUTS and PEGS, however, distinct regions can be identified in the micrographs (**Figure 2**). Both the bromine and PEG terminuses of the other two silanes create non-adsorptive surfaces for ssDNA. We hope that the non-adsorptive regions on the

surface will cause the adsorbed DNA in the surrounding regions to be directed more quickly to binding sites contained there.

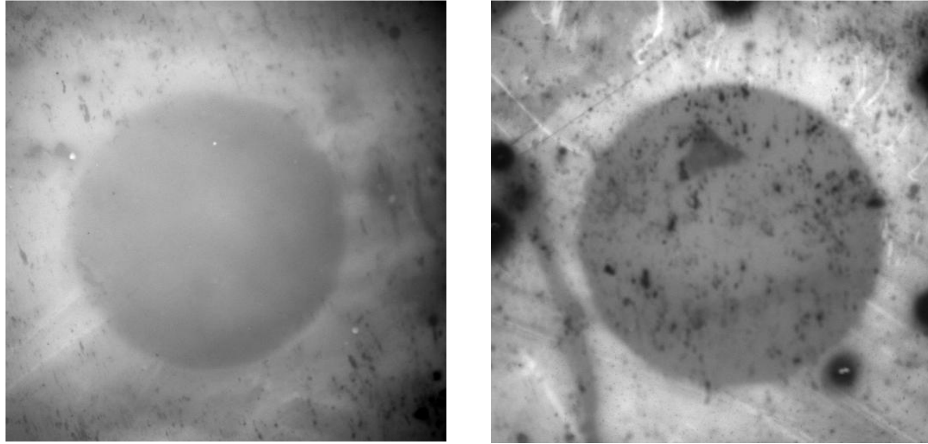

**Figure2: ssDNA adsorbed to BRUTS (L) and PEGS (R)**

In order to identify the conditions affecting an increase in surface diffusion coefficient and capture probability, we have systematically varied the area fraction of backfilled regions, and the chemical makeup of those regions. Implications for the improved design of bioanalytical devices will be discussed.