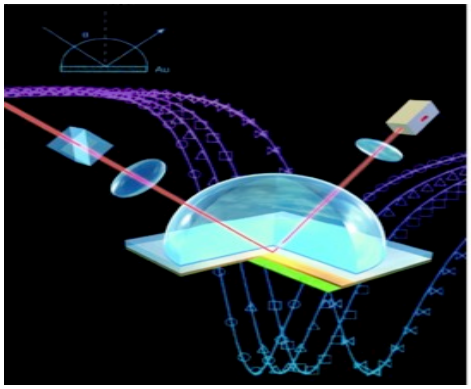
Fabrication of Microarrays With 3' and 5' Thiol-Modifed DNA for Surface Plasmon Resonance Imaging

Introduction

Surface Plasmon Resonance (SPR) imaging is a label free method for monitoring the adsorption of organic molecules and biopolymers proteins onto gold surfaces. DNA microarrays created on gold surfaces can be used for a variety of purposes, including DNA-DNA, RNA-DNA, and protein-DNA interactions at extremely small, nanomolar concentrations [(1)]. The fabrication of microarrays allows for a myriad of applications, including genetic analysis, clinical analysis, and immunoassays. Microarrays provide a simple, rapid, low-cost fabrication method. A continual goal of the Corn Group is to create microarrays that require lower concentrations of compounds for detection.

In Surface Plasmon Resonance, collimated white light is shot through a prism where it hits a gold slide treated with biological molecules. By employing a multiphase complex Fresnel calculation, one can calculate the changes in the index of refraction of ultrathin organic layers on the surface of the slide [(2,3)]. From this, not only can hybridization efficiency of DNA be determined, but the kinetics of biochemical interactions can be found to a precise degree as well.



Objective

Previous experiments by the Corn Group have found that the direction in which thiol-modified DNA is applied onto a microarray affects the signal intensity of SPR measurements by nearly four-fold. DNA applied with the 3' end facing the gold surface of an array produced signals significantly higher than those applied with the 5' end attached, despite the fact that both sequences were identical in their base composition. The goal of this project is not only to determine the cause for such a large difference in signal intensities between 3' and 5' thiol-modified DNA, but also to improve the methodology of constructing DNA microarrays so that smaller concentrations of compounds, such as DNA, are required.

Experimental Design

The following experimental outline was prepared with the help of Dr. Hye Jin Lee (senior staff scientist) and Yuan Li (graduate student). Much of the experimental procedure was also adapted from earlier experiments performed by members of the Corn Group. With the SURP grant, I intend to pursue this project full-time during the summer. Below I have listed the process of creating microarrays and obtaining data from SPR imaging that will be my responsibility over the course of the summer.

a. Preparation of Gold Slides

A thin gold film (45 nm) will be vapor deposited onto a SF-10 glass slide (18 X 18 mm) with an underlying layer of chromium (1 nm) using an evaporator [4,5].

b. Deprotection and Purification of thiol-modified DNA

Thiol-modified DNA purchased from Integrated DNA Technologies contains a thiol group at either the 3' or 5' end. In order to attach the thiol-modified DNA to a cross-linker, the hydrogen of the thiol group must be removed. DNA will be resuspended in a solution of phosphate buffer, pH = 8.4, and subsequently reacted with dithiothreitol (DTT) for 30 minutes. This will remove the hydrogen attached to the sulfur group, leaving it deprotected and available to attach to the cross-linker. Afterwards, the DNA will be purified using High Performance Liquid Chromatography (HPLC). The purified DNA will be dried using a Spin Vac, and then the Optical Density (OD) will be measured using UV/Vis Spectroscopy in conjunction with Ellman's Test. This will allow me to determine the concentration of DNA with an unprotected thiol-group. Because this DNA has free sulfur groups, there is the possibility of disulfide bonds forming between the DNA. Consequently, the purified DNA must be used within a 2-week period.

In my experiment, I intend to use a number of different single stranded DNA sequences as probes. As of right now, the sequences I am in the process of using are listed below:

Name	Sequence
3' thiol modified w3	5' – GTG TTA GCC TCA AGT GTT TTT TTT TTT TTT T(SH) – 3'
5' thiol-modified w3	5' – (HS) TTT TTT TTT TTT TTT GTG TTA GCC TCA AGT G – 3'
3' thiol-modified w1	5' – GTC TAT GCG TGA ACT GTT TTT TTT TTT TTT T(SH) – 3'
5' thiol-modified w1	5' – (HS) TTT TTT TTT TTT TTT GTC TAT GCG TGA ACT – 3'
C 1 (complementary strand to w1)	5' – CAG TTC ACG CAT AGA C –3'
C 3 (complementary strand to w3)	5' – CAC AAT CGG AGT TCA C – 3'

Each of the probe sequences contains a 15-base thymine spacer, which will be used to distance the DNA probe from the surface of the slide, and so that DNA acts more like it is in solution rather than on a surface. I can modify the length of the spacer in later runs of the experiment to determine if the spacer size plays a role in the signal intensity of SPR imaging. Other sequences of DNA will also be used to determine whether the base composition has an effect on the signal intensity. Additionally, the complementary strands of DNA are only perfect matches for the some of the probes that will be implemented onto an array (an array will usually consist of 2 different DNA sequences). This will also allow us to see whether the amount of hybridization affects the signal produced from SPR imaging.

c. Surface Chemistry

Once the gold slides have been created, they will be immersed in a solution of 11-Mercaptoundecylamine (MUAM) for at least 2 hours to create an amine-terminated self-assembled monolayer. Subsequently, the slide will be treated with a solution of 3 mM 9-fluroenylmethoxycarbonyl-N-hydroxy-succinimide (Fmoc-NHS) (3 mM in 1:1 DMSO: 100 mM TEA buffer, pH 7). Excess Fmoc-NHS will be removed from the surface by soaking the slide in DMSO[5].

Afterwards, the gold slide will be photopatterned using UV light from a mercury-xenon arc lamp through a quartz mask for about 1 hour at 400 W. This purges the gold surface of the slide from any of the previous surface chemistry at select spots. A pattern consisting of 750 um square holes will be used for photopatterning, creating a checker-board like pattern.

Once the slide has been photopatterned, it will be soaked in a solution of 1 mM MUAM for at least 2 hours. The cross-linker Sulfosuccinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SSMCC) will then be spotted onto the surface of the slide in 1 mM concentrations. A 1 mM concentration of four types of thiol-modified DNA will be hand-spotted onto a single array, as shown in *Figure (1)*. By using a cross-linker to attach DNA to the array, not only is nonspecific adsorption of DNA onto the gold surface prevented, but also the surface-density of DNA can be manipulated to create optimal conditions.

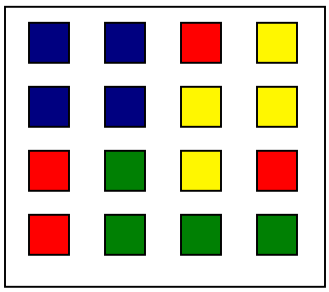


Figure (1): *A layout of where 4 separate DNA probes will be placed on a microarray*

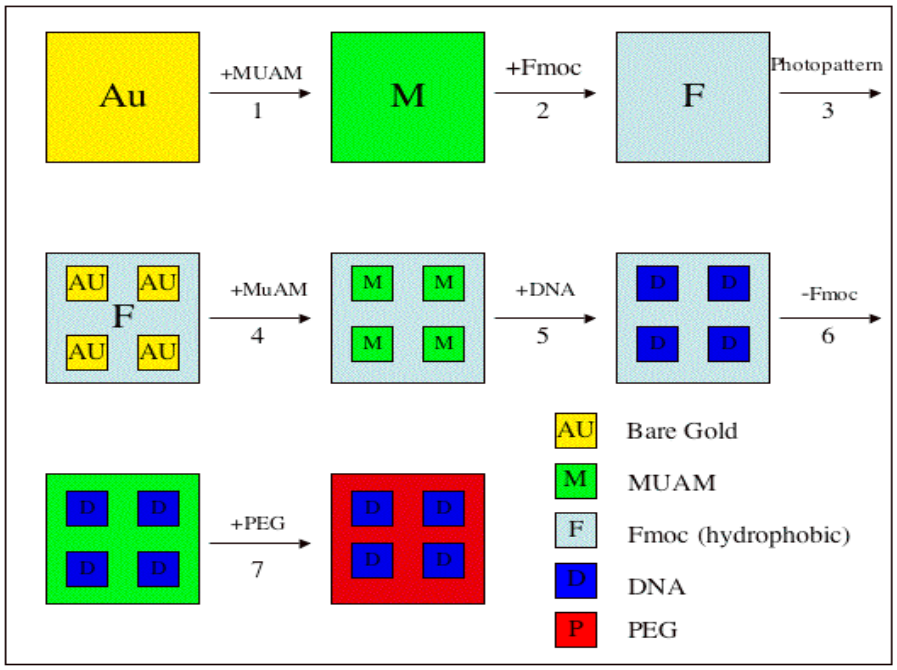
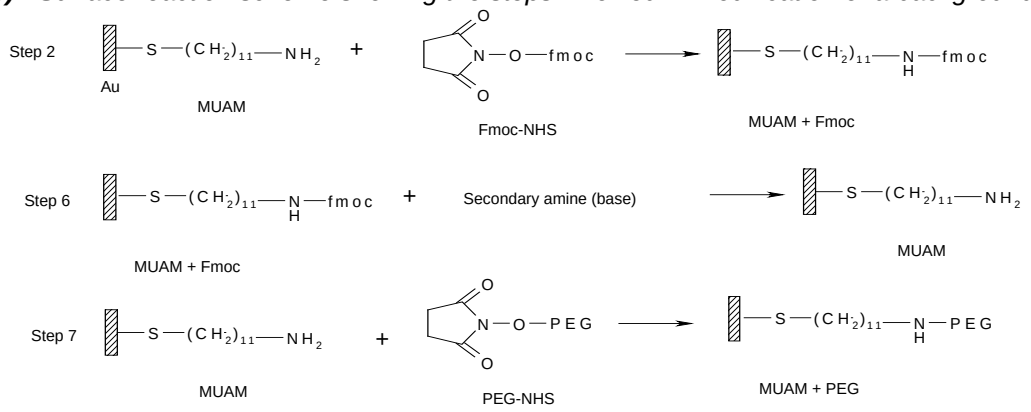


Figure (2): *Fabrication Scheme for the construction of multi-element DNA arrays. Note that this is a simplified scheme, and the one I intend to use for experimentation will consist of 16 probes attached to one surface. A clean gold surface is reacted with MUAM, and subsequently reacted with Fmoc-NHS to*

create a hydrophobic surface. This surface is then exposed to UV radiation through a quartz mask and rinsed with solvent to remove MUAM + Fmoc from specific areas of the surface, leaving gold bare parts. These bare gold areas on the sample are filled in with MUAM, resulting in an array of MUAM pads surround by a hydrophobic Fmoc background. Solutions of DNA are then delivered by pipet onto the specific array locations and are covalently bound to the surface via the bifunctional linker SSMCC. In the final two steps, the Fmoc-terminal gropus on the array background are removed and replaced by PEG groups which prohibit the nonspecific binding of analyte proteins to the background[6].

The surface will next be exposed to a 1 M solution of tris(2-aminoethyl)amine (TAEA), a secondary amine which removes Fmoc from the surface of the array. Finally, the array will be reacted with an NHS ester derivative of poly-(ethylene glycol) (PEG-NHS) to create a background resistant to the nonspecific binding of DNA.

Figure (3): *Surface reaction scheme showing the steps involved in modification of a background array*[6].



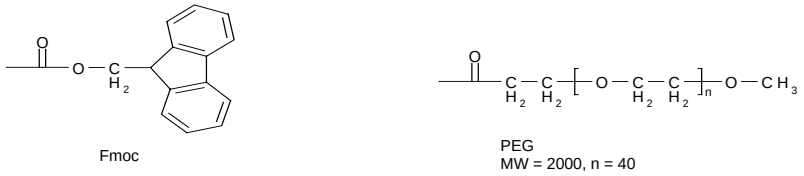
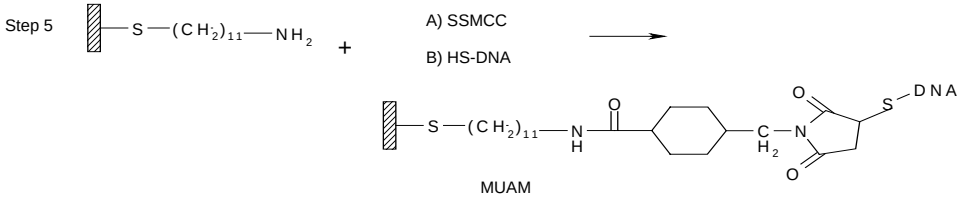
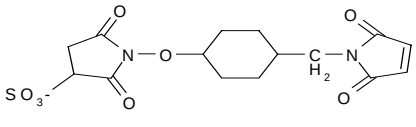


Figure (4)*: Surface reaction scheme showing the immobilization of thiol-terminated DNA to the array surface*





Numerous spectroscopic techniques will be used to characterize the surface attachment chemistries throughout the experiment. Most common will be the use of Polarization Modulation FTIR Reflection-Adsorption Spectroscopy (PM-FTIRRAS) as a means to determine the chemical structure of the self-assembled monolayers during each step of the array fabrication[(7)].

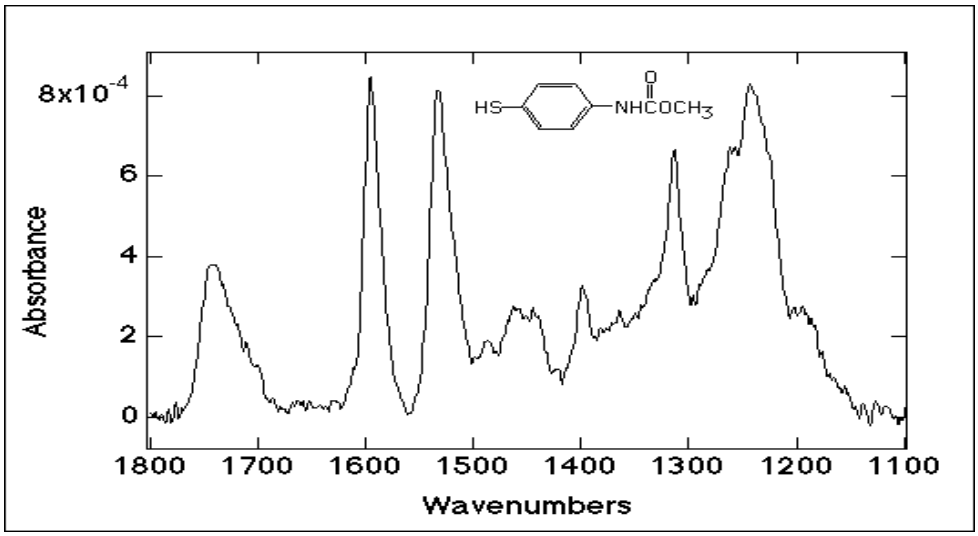


Figure (5): *PM-FTIRRAS spectrum of an alkanethiol monolayer on a vapor-deposited gold film after normalization of data*[(8)]*.*

d. Microchannels

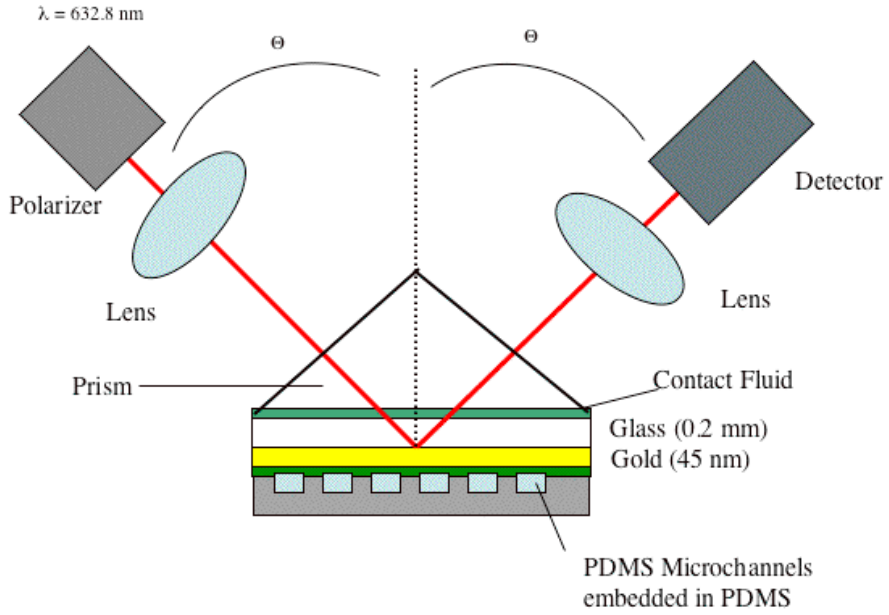
"Microfluidic channels fabricated from poly(dimethylsiloxane) (PDMS) are employed in surface plasmon resonance imaging experiments for the detection of DNA and RNA adsorption onto chemically modified gold surfaces." These microchannels allow one to flow a solution containing complementary strands of DNA over the single stranded DNA probes, resulting in hybridization. PDMS will be placed onto a 3-D Silicon wafer master (created photolithically from a 2-D chrome mask) where it will be cured for 1 hour at 70^{o}C. Subsequently, it will be removed from the Silicon wafer, and the microchannels removed by cutting an 18 X 18 mm section of the polymer containing a set of microchannels[1]. Reservoirs to flow solution through during Surface Plasmon Resonance will be created by cutting out circular ends of each microchannel with a hole punch.

e. SPR Measurements

Once a microarray has been fabricated, I can begin to take measurements using Surface Plasmon Resonance. The microarray is placed in a sample holder in contact with an SF-10 prism. A collimated white light source is shone through the prism, hits the gold surface of the slide, and some of the light is absorbed due to the DNA attached. Solution containing complementary strands of DNA will be flowed through the microchannels in a buffer (pH 7.7) consisting of 20 mM phosphate, 300 mM NaCl, and 1 mM EDTA, allowing the probes to hybridize with the complementary strands. The buffer solution will be introduced to the microchannels via a simple aspiration pumping system.

This hybridization causes a dramatic increase in the absorbance of light, in turn creating a larger signal intensity. This allows us to monitor the hybridization efficiency of the strands of DNA.

Figure (6): *A schematic diagram of an SPR Imager and Microarray.*[2]



References

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Timeline

I intend to work on this project for the remainder of Spring quarter, and given that I receive a SURP Fellowship, full-time during the summer. The following is an expected timeline for the remainder of Spring quarter and Summer:

April

- Preparation of solutions to be used with Microarrays.
- Alignment of SPR Imager
- Alignment of FTIR.

May

- Fabrication of Microarrays containing DNA sequences w1 and w3.
- Fabrication of Microarrays containing DNA sequences w3 and w5.

June

- Fabrication of Microarrays consisting of DNA with Thymine Spacers longer and shorter than 15 bases.

July

- SPR Imaging Measurements using buffer solutions with varying polarities.

August

- SPR Imaging Measurements at various temperatures.