Thursday Afternoon, October 5, 2000

Biomaterial Interfaces
Room 202 - Session BI+NS-ThA

Biosensors
Moderator: A. Chilkoti, Duke University

2:00pm **BI+NS-ThA1 Substrate and Attachment Chemistry Effects on Adsorption and Single-Base Mismatch Discrimination on Immobilized Oligonucleotide Arrays**, ***J.E. Forman***, *L. Gamble, R.S. Gascon, J.I. Henderson, A.D. Suseno, P. Wagner,* Zyomyx, Inc. **INVITED**

Hybridization efficiency and ability to discriminate between perfect match and single-base mismatch target sequences are fundamental to performance of arrays of covalently immobilized nucleic acids (probes). Two factors that significantly contribute to array performance are density and orientation of immobilized probes. Probe density not only controls how much bound target is adsorbed, but also affects the kinetics and thermodynamics of duplex formation. Perturbations to duplex formation occur when the spacing of probes is close enough to force inter-probe association and crowding, phenomena that interfere with target sequence adsorption. However, these perturbations can induce beneficial effects in array performance. For example, crowded nucleic acid surfaces demonstrate lower apparent melting temperatures (T@sub m@'s) than the solution phase, but also show discrimination between matched and mismatched sequences under low stringency conditions. The orientation of the probe is highly dependent on the method of immobilization to the surface. Non-optimal attachment can orient the probe in a way that interferes with duplex formation, or such that it becomes buried within the surface and inaccessible to the target sequence. The substrate used for array preparation ultimately controls both density and orientation; optimization of the substrate can enhance array performance in an assay. Especially interesting is a precise control of probe density, where the density effects alter the T@sub m@'s of immobilized sequences to allow a broad range of sequence to be analyzed in a single temperature assay. We have been exploring a variety of silane modified substrates for the preparation of oligonucleotide arrays, focusing on immobilization of the probe through amine or thiol moieties. The effect of density and attachment chemistry on target adsorption and single-base mismatch discrimination with single and double stranded oligonucleotide target sequences will be presented.

2:40pm **BI+NS-ThA3 Investigation of DNA Hybridization on Surfaces by Surface Plasmon Fluorescence Spectroscopy (SPFS)**, ***T. Neumann***, *W. Knoll,* Max-Planck Institute for Polymer Research, Germany

The investigation of DNA hybridization on surfaces, and as a consequence, the development of DNA-chips and sensors, has been of increasing interest in recent years, since such technology can be used to investigate the human genome. We report here a study of PNA:DNA and DNA:DNA hybridization on surfaces measured by surface plasmon resonance spectroscopy (SPS) coupled with fluorescence (SPFS) and surface plasmon microscopy (SPM). PNA (peptide nucleic acids) and DNA catchers were immobilized either directly on gold surfaces by thiol linkers or via biotin on streptavidin covered gold surfaces. In order to enhance the detection limit of the SPR, the fluorescence signal of dyes attached to the target strands was detected during the hybridization to the immobilized catchers. To get a deeper insight into the underlying principles of the hybridization process near surfaces, conformational changes in the catcher and target DNA structure were monitored by comparing the hybridization kinetics obtained by having the fluorescent dye attached either to the catcher or the target and varying the length of both types of strands. Furthermore by using two different fluorescent dyes on the DNA strands, we were able to carry out Forster transfer experiments during the hybridization step, which allowed us to monitor distance changes between the catcher and target at the surface.

3:00pm **BI+NS-ThA4 Fluorescence Detection of Surface DNA Hybridization Reactions Based on Surface Structural Changes**, ***T.H. Huang***, *S.J. Stranick, M.J. Tarlov,* National Institute of Standards and Technology

We describe a novel fluorescence method for the detection of surface-immobilized DNA hybridization reactions. Solid phase hybridization reactions form the basis of DNA chip technologies that are used for sequencing and genetic diagnostic applications. In conventional fluorescence-based detection schemes, the "target" DNA is typically labeled with a fluorophore. We report a method where instead, the "probe" DNA is labeled with a fluorophore. Our model surface hybridization system uses a mixed monolayer of fluorescein-tagged, thiol-derivatized, single-stranded DNA probes and 6-mercaptohexanol self-assembled on Au surfaces. Prior to hybridization, the fluorophore on the probe is in closer proximity to the gold surface, resulting in greater quenching of the fluorescence signal. Upon hybridization, the double-stranded DNA adopts a rod-like structure that extends the fluorophore away from the gold surface. With the fluorphores located further from the gold surface, quenching is reduced and an increase in fluorescence intensity is observed. Parameters affecting fluorescence intensity such as probe surface coverage, probe length, and target concentration will be discussed. In addition, a comparison of probe- and target-labeled fluorescence detection schemes will be made.

3:20pm **BI+NS-ThA5 Silicon Surface Chemistry for DNA Immobilization**, *T. Strother, Z. Lin, W. Cai, L.M. Smith,* ***R.J. Hamers***, University of Wisconsin, Madison

Many emerging areas of biotechnology, such as "gene chips", seek to leverage many aspects of the existing infrastructure in microelectronics and apply it to new areas. While most previous work has focused on the attachment of DNA to surfaces of gold or glass, we have investigated the chemistry involved in covalently bonding DNA to Si(001) and Si(111) surfaces in a way that retains its ability to selectively hybridize with its solution-phase complements. The use of XPS to study the chemical structure at each step in the DNA attachment process has lead to the development of new procedures that are both simple and robust. Starting with hydrogen-terminated Si(001) and Si(111) surfaces, photochemical excitation at 254 is used to remove the photo-labile hydrogen; the exposed surface is then reactive toward organic molecules containing one or more unsaturated C=C bonds. "Linker" molecules containing a C=C double bond with another functional group(such as an amine or ester group) are then used to provide a dense set of surface-bound functional groups for attachment of DNA. To control the selectivity of the attachment process, however, careful optimization of the molecular structure of the linker and the other processing conditions are required. The density and chemical uniformity of the surface (as judged by XPS) is highly correlated with the intensity and selectivity achieved in subsequent binding of the surface-bound DNA to its fluorescently-labeled complements in solution. The results show that control of surface chemistry indeeds leads to significant improvements in the formation of DNA-functionalized silicon surfaces.

3:40pm **BI+NS-ThA6 BARC: A Magnetoresistive Biosensor@footnote 1@**, ***P.E. Sheehan***, Naval Research Laboratory*; R.L. Edelstein,* Geo-Centers, Inc.*; C.R. Tamanaha, M. Miller,* Naval Research Laboratory*; L. Zhong,* Geo-Centers, Inc.*; R.J. Colton, L.J. Whitman,* Naval Research Laboratory

The Bead ARray Counter (BARC) is a revolutionary biosensor that uses DNA microarrays, magnetic microbeads, and giant magnetoresistive (GMR) magnetic field sensors to detect and identify biological molecules.@footnote 2@ The current prototype is a table-top instrument with integrated fluidics under development for the detection of biological warfare agents. The core of the sensor is a small, microfabricated chip containing a GMR sensor array for detection of up to eight different pathogens. Oligonucleotide probes complementary to pathogen target sequences are arrayed onto the microfabricated chip directly above the GMR sensors. Specific hybridization is measured and discriminated from non-specific background by addition of functionalized magnetic microbeads that bind to the captured target DNA. The beads tethered to the surface are detected by the GMR sensors, with the intensity and location of the signal indicating the concentration and identity of the target pathogens. A complete assay, including hybridization and detection can be performed in approximately 30 min. Because each GMR sensor is capable of detecting a single magnetic bead, in theory, the BARC biosensor should be capable of detecting a single molecule. With recent advances in GMR technology for computer memory, chips with millions of sensors will soon be commercially available, enabling the development of a BARC sensor capable of detecting thousands of analytes simultaneously. We will discuss the scientific and technical challenges to making such a sensor system a reality. @FootnoteText@ @footnote 1@Supported by the Defense Advanced Research Projects Agency. @footnote 2@Edelstein et al., Biosensors & Bioelectronics 14, 805 (2000).

4:00pm **BI+NS-ThA7 A Biosensor Based on Force Differentiation@footnote 1@**, ***C. Yanavich***, *M. Malito,* Nova Research, Inc.*; G.U. Lee, L.J. Whitman, R.J. Colton,* Naval Research Laboratory*; M. Natesan,* Geo-Centers, Inc.

We are developing an array biosensor that uses a magnetic force to differentiate specific ligand-receptor binding from non-specific ligand-surface binding. In this force differentiation assay biosensor, capture antibodies that will bind to specific target analytes within a sample are first

coated onto a surface. The captured analyte is then sandwiched by a second antibody that is attached to a magnetic microbead. A magnetic force with well-defined magnitude and orientation is applied to remove the non-specifically adsorbed beads, and a semi-automated optical reader then measures the number of the specifically bound beads remaining on the surface (which can be correlated with the analyte concentration). The original prototype used polystyrene microtitre wells for multi-analyte detection. We are now developing a second-generation sensor that employs a filter membrane as the capture surface. The filter facilitates concentration of the antigen on the surface and enhances the antigen-antibody interactions, significantly reducing the assay time (to ~30 min), and increasing the sensitivity by two-to-three orders of magnitude. Several techniques are being investigated to pattern capture antibodies onto the filter surface in order to enable multi-analyte detection on a single disposable filter. They include photo patterning with photo-activated biotin or caged photo-biotin, and imprinting via PDMS masks. We will also discuss our development of alternate techniques aimed at simplifying the bead counting system. @FootnoteText@ @footnote 1@Supported by the Joint Service Technical Panel for Chemical and Biological Defense (JSTPCBD).

4:20pm **BI+NS-ThA8 Encapsulation of Smart Polymers in Silica: Stimuli-Responsive Porous Hybrid Materials That Incorporate Molecular Nano-Valves**, *G.V. Rama Rao,* ***G.P. Lopez,*** University of New Mexico*; A. Chilkoti,* Duke University

Elastin (a protein) and poly(N-isopropyl acrylamide) (PNIPAAM, a synthetic polymer) are two types of thermo-sensitive, smart polymers which exhibit inverse solubility in water upon heating and undergo the transition from hydrophilic conformations to hydr ophobic conformations at a temperature known as lower critical solution temperature (LCST). This interesting property has led to have several applications in biotechnological areas. In this report, we describe the development of molecular switches as nan os copic actuators that can control the transport of chemical species by encapsulating PNIPAAM and elastin in dense silica gels by sol-gel synthesis. Cycling through the LCST can control molecular permeability through these hybrid materials. The pores res ult ing from the transition can selectively transport molecular species depending on their size. For example, permeation experiments revealed the LCST behavior of PNIPAAM in silica-polymer membranes and was identified to be 31 °C. DSC studies on bu lk gels are in good agreement with the permeation results. Cycling of the membranes between 25 and 40 °C indicates the membranes possess reversible, variable permeability while maintaining good mechanical stability. Importantly, permeation experiments on PNIPAAM-si lica membranes with various molecular weights of poly(ethylene glycol) have clearly demonstrated that the membrane is acting as a molecular switch by being impermeable below the LCST, and permeating the lower molecular weights of poly(ethylene glycol) and filtering out higher molecular weight polymers above LCST.

4:40pm **BI+NS-ThA9 Adsorption Behavior and Optical Properties of Surface-Adsorbed Polystyrene Nano Particles**, ***M. Himmelhaus,*** Universität Heidelberg, Germany*; H. Takei,* Hitachi Central Research Laboratory, Japan

Polystyrene (PS) nano particles have become popular tools in photonics, nano technology, and life science since they have become commercially available in a wide range of sizes with narrow size distribution. While most applications utilizing surface-adsorbed PS nano particles deal with ordered arrays on mesoscopic scale, recently a chemically induced method for adsorption of such particles was introduced to yield random-close-packed (rcp) monolayers of almost arbitrary lateral extension. Such layers can be used as a template for the formation of cap-shaped Gold nano particles that exhibit extraordinary optical properties@footnote 1@ and thus can be developed into a sensitive optical biosensor.@footnote 2@ Here we demonstrate that the chemically induced adsorption method can be combined with alkanethiol chemistry to gain better control of sphere adsorption. Thus, sphere layers of varying density can be fabricated and their optical properties can be studied as a function of coverage. By further utilizing Micro Contact Printing (µCP) of tailgroup modified alkanethiols 2D patterns of rcp PS sphere monolayers with a lateral resolution of a few microns and a total pattern area of ~1 cm@super 2@ can be produced. These patterns are a first step to the development of an optical biosensor based on cap-shaped Gold nano particles with massively parallel detection capability. @FootnoteText@ @footnote 1@ H. Takei, J. Vac. Sci. Technol. B 17 (5) 1906, 1999 @footnote 2@ M. Himmelhaus, H. Takei, Sens. Acuators B 63 (1-2) 24, 2000

5:00pm **BI+NS-ThA10 Formation and Patterning of Supported Fluid Lipid Bilayers on a High Refractive Index Substrate**, ***C.M. Ajo,*** *L.C. Kam, S.G. Boxer,* Stanford University

Supported lipid bilayers are a useful model system to probe cellular membrane components and their interactions in a near native environment. Specifically, membrane components reconstituted in supported lipid bilayers create a well-defined two-dimensional system that can be manipulated - and then interrogated with a variety of surface specific and optical techniques. Several of these techniques rely on evanescent fields to probe the region near the solid support-lipid bilayer interface. However, the solid support typically has been a low refractive index material that permits the evanescent wave to penetrate significantly beyond the bilayer (650 Å). Here we report the formation of supported lipid bilayers on lithium niobate (LiNbO@sub 3@), a material with a high refractive index (n=2.3). Vesicle fusion onto lithium niobate forms a single uniform supported lipid bilayer that exhibits lateral diffusion properties similar to glass-supported lipid bilayers. By blotting and stamping,@footnote 1@ supported bilayers can be patterned reversibly, and the lipid components reorganize in response to an electric field. The high refractive index of lithium niobate restricts the penetration of an evanescent field to within 160 Å of the solid support-lipid bilayer interface. This provides a method to study the cell-supported lipid bilayer interface, since the relevant distances are on this order. Additionally, the transparency of lithium niobate ove a wide range of wavelengths makes it a useful substrate for both visible and infrared studies. @FootnoteText@ @footnote 1@J. S. Hovis and S. G. Boxer, Langmuir 16, 894 (2000).

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