## Biomaterial Interfaces
## Room 318/319 - Session BI+SS-ThA

## Biodiagnostics
**Moderator:** G. Leggett, University of Sheffield, UK

2:00pm **BI+SS-ThA1 Engineered DNA and RNA Molecules as Biochemical Tools**, ***R. Breaker,*** Yale University **INVITED**

RNA and DNA molecules can be engineered to perform as precision allosteric enzymes, or "molecular switches", that are modulated by specific effectors. These designer sensor elements have numerous applications ranging from the construction of biosensors to the development of novel genetic switches. We have embarked on a program to establish effective molecular engineering strategies for switch construction, and to establish the fundamental principles that dictate the performance characteristics of these molecules. In pursuing our objectives, we have created a variety of RNA molecular switches that are modulated by specific target molecules that range from nucleotides and oligonucleotides to drug compounds, metabolites and toxins. In addition, we have begun to explore the use of immobilized RNA switches for the construction of advanced biosensor arrays. Our findings suggest that RNA and DNA have a significant untapped potential for functioning as precision molecular switches in both industrial and natural settings.

2:40pm **BI+SS-ThA3 Base-dependent Competitive Adsorption of DNA on Gold**, ***D.Y. Petrovykh,*** University of Maryland and Naval Research Laboratory*; H. Kimura-Suda, M. Tarlov,* National Institute of Standards and Technology*; L.J. Whitman,* Naval Research Laboratory

We characterize the room-temperature adsorption of single-stranded DNA (ssDNA) homo-oligonucleotides from solution onto polycrystalline Au films, including competitive adsorption between all possible pairs of unmodified oligomers. Although recent studies have shown that different DNA bases and homo-oligonucleotides interact differently with Au surfaces, competitive interactions among the bases - which will occur in most practical applications - have not been systematically addressed. We use Fourier transform infrared (FTIR) and X-ray photoelectron (XPS) spectroscopy to characterize the resulting films, and observe that oligonucleotides adsorb with a strongly base-dependent affinity, adenine (A) > cytosine (C) >= guanine (G) > thymine (T). In competitive adsorption experiments on Au, oligo(dA) strongly dominates over the other oligonucleotides. The relative adsorption affinity of oligo(dA) is so great that it competes effectively against adsorption of thiolated oligomers, and even causes hybridized oligo(dA)*oligo(dT) duplexes to denature in the presence of Au. The asymmetric adsorption affinities of the oligonucleotides must be carefully considered in systems using gold substrates, electrodes, or nanoparticle labels, and are likely to also occur on other substrates of practical importance.

3:00pm **BI+SS-ThA4 MALDI MS of Proteins Separated on a Chemical Gradient Modified Open Channel Microchip**, *G.R. Kinsel,* ***X. Li,*** *R.B. Timmons,* University of Texas at Arlington

Efficient methods for protein separation and characterization are critical to the success of a wide array of biological and biomedical research activities. Current methods involve electrophoretic separation of proteins, followed by staining, excision, digestion and analysis of isolated proteins by Matrix-Assisted Laser Desorption / Ionization Mass Spectrometry (MALDI-MS). This approach is both time consuming and subject to significant protein loss resulting from the various manipulations of the sample. Research in our laboratory is directed at circumventing these limitations through the incorporation of the sample separation process directly on the surface of the MALDI-MS sample stage. In our approach substrates suitable for use as the MALDI sample stage are modified to incorporate open electrophoretic separation channels. Substrates that have been employed include PMMA chips, which are patterned using heat-imprinting methods, and silicon wafers, which are patterned using conventional plasma etching methods. A chemical gradient is developed along the separation channel by masking adjacent areas and sequentially depositing thin films on the channel via pulsed RF plasma polymerization of allyl alcohol at various duty cycles. Control mixtures of peptides having varying hydrophilicity are electrokinetically injected into the gradient chemically modified open channel, eletrophoretically separated and then analyzed by rastering the MALDI desorption laser across the channel while acquiring MALDI mass spectra. Successful results obtained to date, demonstrate the potential value of this approach for improving sensitivity and specificity in MALDI MS analysis.

3:20pm **BI+SS-ThA5 Pb@super 2+@ Sensitive Catalytic DNA Assay Integrated into Microfluidic Channels**, ***R.A. Zangmeister,*** *M. Tarlov,* National Institute of Standards and Technology

Advances in microchip technology coupled with innovative bioassays are advancing the field of biosensors. We previously reported a method for immobilizing single-stranded DNA (ss-DNA) probe molecules in polyacrylamide hydrogels within plastic microfluidic channels. Spatially defined plugs are formed by photopolymerization of a solution containing 19:1 polyacrylamide/bisacrylamide and ss-DNA modified at the 5' end with an acrylic acid group. Low concentrations of fluorescent-tagged ss-DNA targets can be captured and detected in the hydrogels. We aim to couple this technology with a novel bioassay based on the response of catalytic DNA to Pb@super 2+@ ions in solution. It is reported to show > 80-fold selectivity for Pb@super 2+@ over other divalent metal ions, and with fluorescent tag modifiers can be used to detect Pb@super 2+@ ions over a large concentration range (10 nmol to 4 mmol).@footnote 1@ Our goal is to immobilize the enzyme strand sequence of the catalytic DNA duplex into the hydrogel plugs immobilized in microfluidic channels. Our strategy is to electrophorese fluorescently tagged substrate strands into the hydrogel plug where they hybridize with the immobilized enzyme strand to form the catalytic DNA system. Then Pb@super 2+@ is electrophoresed into the hydrogel plug resulting in the catalytic cleavage of the substrate strand and the release of the fluorescent-tagged sequence fragment that is detected using a fluorescence microscope. The combination of these two technologies results in a Pb@super 2+@ detection system with enhanced sensitivity due to the high loading of DNA probes in the hydrogel plug, the spatially confined, directed mass transfer characteristics of the microfluidic channels, and the inherently low fluorescent background of the hydrogels. The immobilization, retention of catalytic DNA activity, and current limits of detection will be discussed. @FootnoteText@@footnote 1@ Li, J.; Lu, Y., J. Am. Chem. Soc. 2000, 122, 10466-10467.

3:40pm **BI+SS-ThA6 Monitoring Neurotransmitters with Voltammetry**, ***R.M. Wightman,*** University of North Carolina **INVITED**

Carbon-fiber microelectrodes can serve as chemical sensors for the detection of easily oxidized chemical messengers such as dopamine, serotonin, and histamine in biological systems. The electrodes have micron dimensions and can be used on millisecond time scales. Thus, they can be used to measure neurotransmitter release at the level of single cells or in the brain of intact, behaving animals. Such measurements are giving new insights into the complex chemical interactions that regulate behavioral states.

4:20pm **BI+SS-ThA8 Adsorption Behavior of Proteins in Microcapillaries**, ***A. Bhattacharyya,*** *K. Lenghaus, D. Halagowder, J.J. Hickman,* Clemson University*; J.W. Jenkins, S. Sundaram,* CFD Research Corporation

The dynamics of protein adsorption, desorption and denaturization are important factors in determining the efficacy of a microfluidic device for biotechnology applications. When a protein solution is passed through a microcapillary, the protein molecules can adsorb onto the surface of the capillaries and can often subsequently denature. Hence an understanding of the adsorption behavior of a protein is very important in order to determine the basic parameters for fabrication of a microfluidic based MEMS device. Most of the research on protein adsorption characteristics is based on static systems. However, the adsorption behavior of proteins in static and flow systems is not necessarily the same. Our research focuses on investigating the difference in the adsorption behavior of proteins under flow and static conditions, using enzymatic proteins as probes. We have used enzymes such as alkaline phosphatase, glucose oxidase and horseradish peroxidase in our studies. The microcapillaries used were PEEK (Poly-Ether-Ether-Ketone) and PTFE (Polytetrafluoroethylene). A total protein assay (MicroBCA) was used to quantitate the amount of protein adsorbed to the surface and enzymatic assays were used to estimate the activity of the proteins. A statistical model based on the Langmuir equation was used for extracting the kinetic binding constants and the protein coverage on the surface. Our results indicate that there is a significant difference in the surface affinities and binding site densities observed in static and flow conditions. These results will enable us to improve existing protein adsorption and fluid dynamics software and eventually create design rules for biocompatible MEMS devices.

4:40pm **BI+SS-ThA9 Micro- to Nanofluidic Systems for Bioanalysis**, ***G.P. Lopez***, University of New Mexico; *S.S. Sibbett,* Intel Corp.; *D. Petsev,* University of New Mexico; *C.F. Ivory,* Washington State University; *M. Piyasena, A. Garcia, L.K. Ista, M.J. O'Brien, P. Bisong, S.R.J. Brueck,* University of New Mexico

This talk will present an overview of efforts at the University of New Mexico to develop chip based micro- and nanofluidic systems for biosensing and bioseparations. Microfluidic systems to be described include microchip countercurrent electroseparation (in collaboration with Intel Corp.) and affinity microcolumns with fluorescence detection. New methods for fabrication of nanofluidic systems based on interferometic lithography will also be described. These methods are especially well suited for manufacture of bioanalytical systems that incorporate large scale integrated nanofluidic components. Characterization and modeling of fluidic properties of the bioanalytical systems will be emphasized.

5:00pm **BI+SS-ThA10 Chitosan - A Biomaterial Interface that can be Selectively Deposited onto Micropatterned Surfaces and Conjugated to Sensing Biomolecules**, ***L.-Q. Wu***, *H. Yi,* University of Maryland Biotechnology Institute; *M.J. Kastantin, S. Li, D.A. Small, J.J. Park,* University of Maryland; *T. Chen,* University of Maryland Biotechnology Institute; *G.W. Rubloff, R. Ghodssi,* University of Maryland; *W.E. Bentley, G.F. Payne,* University of Maryland Biotechnology Institute

We are examining the amino-polysaccharide chitosan as a biomaterial interface. Chitosan's pH-dependent electrostatic properties allow it to be selectively deposited (i.e. "templated") onto micropatterned electrodes in response to an applied voltage. Deposition of chitosan, or chitosan-containing conjugates, is rapid (about 2 minutes) and can be performed under mild conditions. After neutralization, the films are retained without the need for an applied voltage. These films can also be removed from the electrode using mildly acidic conditions (pH<6). Chitosan's amine groups are also nucleophilic and can readily react with a variety of reagents. In particular, standard coupling chemistries can conjugate proteins and oligonucleotides with chitosan. We are exploiting glutaraldehyde coupling chemistries to anchor nucleic acids and proteins onto chitosan surfaces. In one study, we tethered oligonucleotide probes onto an electrochemically deposited chitosan surface and examined the bio-detection of mRNA by a hybridization-based assay. In a second study, we selectively deposited chitosan on an electode surface embedded in the base of a microfluidic channel. The green fluorescent protein (GFP) was subsequently anchored to this chitosan surface. In summary, chitosan is unique interface biomaterial - it can be templated onto a microfabricated surface and conjugated to bio-molecules. We are currently exploiting these capabilities in biosensor and bio-MEMS applications.

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