Tuesday Afternoon, November 5, 2002

Biomaterials
Room: C-201 - Session BI+SS-TuA

Molecular Recognition Surfaces
Moderator: M.J. Tarlov, National Institute of Standards and Technology

2:00pm **BI+SS-TuA1 Medard W. Welch Award Address: The Biointerface Examined in Five Dimensions**, ***B.D. Ratner****, University of Washington **INVITED**

Twenty years ago, we had no such word as "biointerface." Now we use that word almost routinely to suggest surface-localized events between biological systems and solid surfaces (solids that vary in solidity from almost fluid to hard). To examine with a new perspective this burgeoning field, five facets of the biointerface will be explored. The five faces of the biointerface will be: temporally (and historically), spatially, molecularly, entrepreneurially and virtually (in computer space). Studies from our groups at the University of Washington and from others in the field will be presented. The talk will aim at defining the new field of the biointerface, relating it to "classical" surface science and highlighting opportunities.

2:40pm **BI+SS-TuA3 "Smart" Biomolecular Conjugates**, ***P.S. Stayton****, A.S. Hoffman, N. Malmstadt, C. Hu, S. Kulkarni*, University of Washington

One of the hallmarks of biological systems is their ability to change important properties in response to environmental cues. We have been developing stimuli-responsive biomolecular materials for biosensors, diagnostics, affinity separations, microfluidic devices, and chip/array devices that exhibit responsiveness to specific environmental cues. For many of the diagnostic and sensor technologies that utilize biomolecular recognition properties, there is a continuing need for better control routes. Current environmental methods are relatively harsh and can lead to damage of biomolecules and cells. In addition, the environmental signals are typically large general solution changes and thus not targeted to selective recognition components. The stimuli-respon sive biomolecular materials allow reversible control over protein recognition properties by utilizing small changes in environmental conditions or signals. The "smart" polymers reversibly cycle between an extended and hydrophilic random coil, and a collap sed, hydrophobic state that is reduced in average volume by ca. 3-fold. When the smart polymers are attached at defined protein side-chains, typically by genetically engineering cysteine or lysine residues, the polymers serve as sensors and actuators to c ontrol access of ligands or su bstrates to binding or catalytic sites. This general approach targets mild environmental signals to specific polymer-protein conjugates, and thus for example allows differential control of different antibodies in a device by using conjugated polymers that are sensitive to different signals (e.g. antibody 1 with pH, antibody 2 with temperature, antibody 3 with light). They can thus allow multiplexing control in complex mixtures, and are thus relevant to a number of different diagnostic and sensor formats.

3:00pm **BI+SS-TuA4 Molecular Recognition Mediated Fabrication of Protein Nanostructures by Dip-Pen Lithography**, *J. Hyun, S.J. Ahn, W. Lee,* ***S. Zauscher****, A. Chilkoti*, Duke University

The spatially controlled immobilization of biomolecules on solid surfaces at the nanometer length-scale is driven by the possibility of fabricating new sensors and actuators that will enable detection and actuation at the single molecule level. This communication describes how dip-pen nanolithography (DPN) in combination with the high-affinity streptavidin-biotin, protein-ligand system provides a simple and versatile "bottom-up" approach to create nanoscale biomolecular architectures in a step-wise fashion. This method involves the fabrication of nanoscale features by patterning a self-assembled monolayer (SAM) of a COOH-terminated alkanethiol on a gold substrate by DPN, followed by covalent immobilization of a high-affinity small-molecule ligand (biotin) onto the nanopatterned SAM and subsequent molecular recognition of its protein binding partner (streptavidin) from solution. We fabricated streptavidin nanostructures with lateral feature sizes in the range of 10-400 nm by this method, and have shown that the streptavidin nanopatterns can be used as a template to pattern biotinylated molecules of interest from solution. Because the binding of the final, target molecule is mediated by a highly specific molecular recognition interaction that occurs solely in the patterned region against a non-fouling background, this approach should allow patterning a biomolecule of interest directly from complex mixtures such as cell lysate without purification, which is not possible with alternative DPN methods that involve physisorption or covalent conjugation.

3:20pm **BI+SS-TuA5 Threading DNA Through a Nanopore: Applications for Analyte Detection**, ***J.J. Kasianowicz****, S.E. Henrickson, B. Robertson,* National Institute of Standards and Technology, *H.H. Weetall,* EPA, *M. Misakian,* National Institute of Standards and Technology **INVITED**

We recently demonstrated that single-stranded DNA (ssDNA) can be driven electrophoretically through a solitary Staphylococcus aureus alpha-hemolysin (alpha-HL) ion channel. In an effort to use this model system to understand DNA transport in biological systems, we show that the partitioning of ssDNA into the pore depends on the side to which the polymer is added and on the magnitude of the applied potential. Kramer's reaction rate theory was used to estimate both the height of the energy barrier for polymer translocation and the integral number of charges on ssDNA that interact with the barrier. In a related research effort, we illustrate three experimental results that suggest the interaction between polymers and a single nanopore can be used to quantitate analyte concentration and type. First, the probability that ssDNA enters the alpha-HL channel is proportional to the polymer concentration. Second, analyte binding to sites on ssDNA predictably alters the ability of the polymer to thread through the pore. Third, different ssDNA homopolymers induce current blockade patterns that are characteristic of the nucleotide type. We compare this method to other channel-based detection schemes. Finally, we show that modified polynucleotides might prove useful as "molecular rulers" for probing the structure of nanopores.

4:00pm **BI+SS-TuA7 Novel Immunosensor Interfaces based on Mixed Self-Assembled Monolayers of Thiols on Gold**, ***F. Frederix****, M. Boesmans, K. Bonroy, W. Laureyn, A. Campitelli,* IMEC, Belgium, *M.A. Abramov, W. Dehaen, G. Maes,* KULeuven, Belgium

The two components that make up a biosensor are the biological recognition layer, which selectively binds the analyte, and the transducer which translates this recognition event into an electrical signal. The increasing miniaturization of biosensor transducers (and thus of their active areas) and the demand for sensitivity, require a fully evaluated and optimized covalent immobilization of antibodies. Our research is therefore not only focusing on the transducer but also on the biological interface. This biological recognition layer mainly determines the specificity, stability, reproducibility, and durability of the biosensor as a whole. Our strategy is to achieve the above-mentioned properties based on mixed Self-Assembled Monolayers on gold. The realization of a biological recognition interface encompasses various aspects. Cleanliness and structural properties of the gold surface are very important for perfect SAM formation and were therefore optimized. Novel thiols able to couple antibodies or to mitigate non-specific adsorption were synthesized and evaluated, along with new molecules for blocking. The mixed monolayer formation of these novel thiols was characterized using contact angle measurements, XPS, cyclic voltammetry, and GA-FTIR. The immobilization of proteins on mixed SAMs is the most important step in the realization of immunosensors because it determines the activity of the antibodies and therefore the sensitivity. Random and orientated immobilizations of (chemically modified) antibodies on mixed monolayers of thiols were compared using Surface Plasmon Resonance. The enhanced sensitivity (< 0,1 ng/mL) and selectivity (no non-specific adsorption) were compared to commercially available biological recognition layers. In summary, we will show the importance of the biological recognition layer for the global performance of a biosensor and how the sensitivity can be drastically enhanced by modifications on the biological interface of an immunosensor.

4:20pm **BI+SS-TuA8 Electrostatic and Fluorescence Sensing of DNA Hybridization at Electrode Surfaces**, ***R.M. Georgiadis****, J. Wang, L.K. Wolf, A.W. Peterson,* Boston University

Current microarray technologies, based on specific probe-target hybridizations, often suffer from nonspecific surface interactions. In addition, for surface immobilized probes, thermodynamic equilibrium conditions may not be reached without excessively long incubation times and hybridization may be kinetically or sterically inaccessible for some probe sequences or for some surface probe densities. In previous work on perfectly matched duplexes, we have shown that probe density is a controlling factor for DNA hybridization at surfaces. Here, we expand our studies to investigate probe density effects for mismatched sequence or targets that access different binding locations on the immobilized probe. To improve mismatched hybrid discrimination we detect different dissociation

***** Medard W. Welch Award Winner

profiles for matched and mismatched 25-mer targets from surface-immobilized probes in the presence of an applied repulsive electrostatic field and present denaturation profiles for surface-bound hybrids obtained by continuously varying the applied electrostatic surface field. Finally, we examine the immobilization and hybridization of covalently-bound molecular beacons on gold surfaces using surface plasmon resonance (SPR) spectroscopy and fluorescence spectroscopy.

4:40pm **BI+SS-TuA9 Characterization of DNA on Gold: A Quantitative Surface Science Approach**, ***D.Y. Petrovykh***, University of Maryland - College Park / NRL, *H. Kimura-Suda, M.J. Tarlov*, National Institute of Standards and Technology, *L.J. Whitman*, Naval Research Laboratory

Covalent attachment of thiolated DNA onto gold surfaces is one of the most common methods for immobilizing aqueous DNA onto solid substrates. The formation of the DNA film in this case is thought to closely resemble that of alkanethiol self-assembled monolayers (SAMs). DNA films in modern applications, e.g. DNA microarrays, are < 10 nm thick with submonolayer surface coverage, which means that the traditional surface characterization techniques can be employed to complement biochemical analysis. We are applying XPS, FTIR, and ellipsometry to systematically quantify the chemical structure and coverage of self-assembled single-stranded DNA (ssDNA) films. Thymine (T) has the simplest structure of the four nucleotide bases: a single ring with two N atoms. Moreover, the environment of the two N atoms is so similar that the resulting XPS peak is consistent with a single N1s state in a polymer-like material. Backbone P atoms produce a base-independent single P2p peak. N and P are not subject to significant contamination, so the peak intensities can be used to estimate the coverage of immobilized DNA. N1s chemical shifts together with the base-dependent N/P ratio can confirm the presence of specific polynucleotides on the surface. The coverage determined by XPS is linearly correlated with base-specific IR features and agrees with absolute values obtained from radiolabeling measurements. We will also discuss how the XPS and IR spectra of dT-polynucleotide films can provide information about other basic properties of ssDNA films, such as uniformity and orientation, as well as issues of damage, degradation and contamination.

5:00pm **BI+SS-TuA10 Antibacterial Coatings of Immobilised Furanones**, ***H.J. Griesser***, *S. Al-Bataineh*, University of South Australia, *B.W. Muir, H. Thissen, M. Willcox*, CRC for Eye Research and Technology, Australia

The formation of bacterial biofilms and subsequent infections can cause serious complications in the use of biomedical devices such as catheters, and broadly effective technology is lacking. Nature has, however, addressed very effectively the problem of microbial colonisation of surfaces. For instance, the red alga Delisea pulchra secretes brominated furanones that prevent its microbial colonisation. These compounds are thought to interfere with bacterial quorum sensing by their chemical similarity with homoserine lactone, an important bacterial regulator. We have immobilised various furanones onto synthetic surfaces and tested the efficiency of such coatings in bacterial colonisation assays. A broadly applicable covalent immobilisation strategy involves nitrene chemistry, with light-activated reaction between furanones and azido aniline coupled onto a surface hydrogel interlayer. This allows coupling of furanones without reactive substituents but is non-selective with regard to molecular orientation and location of attachment. Other strategies require functionalised furanones, for example reaction between a hydroxylated furanones and surface isocyanate groups; such furanones can be more difficult to synthesize. Work to date has produced substantial reductions in bacterial colonisation, but not to the high degree required in clinical applications. Investigations now focus on the interactive effects of furanone molecular composition, immobilisation chemistry and surface density. An interesting finding is that these compounds are effective when surface immobilised although the classical microbiological model of homoserine lactone action requires entry into the microbial interior. This dichotomy calls for detailed surface characterisation of furanone coatings, including study of whether the entire population of surface-bound molecules is indeed covalently linked and thus non-diffusible and acting via a different mechanism to stifle bacterial colonisation.

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