Tuesday Morning, November 3, 1998

Biomaterial Interfaces Group
Room 326 - Session BI-TuM

Biosensor-Biology Interface

Moderator: B.D. Ratner, University of Washington

8:20am **BI-TuM1 Gated Ion Channel Devices within Synthetic Tethered Membranes**, ***B.A. Cornell***, *L. Braach Maksvytis, L. King, P. Osman, B. Raguse, L. Wieczorek,* Cooperative Research Centre for Molecular Engineering, Australia*; R. Pace,* Australian National University, Australia **INVITED**

A novel biosensor based on switching the conductance of a population of molecular ion channels is described. . The active elements of the ion channel switch comprise a gold electrode to which is tethered a lipid membrane containing ion channels linked to an appropriate receptor. The approach can be used with most types of receptor including antibodies and nucleotide probes. In its simplest form the technique is sensitive to below 1 picoMolar concentrations of proteins in 5 minutes. The sensor can readily be incorporated as an integral component of a microelectronic circuit and can used in multi-element arrays. It has a wide range of applications and operates in complex media including whole blood. Examples include: cell typing, the detection of proteins, bacteria, viruses, antibodies, DNA targets, electrolytes, drugs, pesticides, heavy metals and other high and low molecular weight compounds. The device has been used to detect the equilibrium and kinetics of the binding of analytes from solution, the kinetics and number association of molecules at the surface of the tethered membrane and the kinetics and conduction of ion channels within the membrane. A description will be given of the assembly and characterisation of the device over a range of applications.

9:00am **BI-TuM3 Interaction of Lipid Vesicles and Cell Membranes with Alkylthiol Monolayers**, ***A.L. Plant***, National Institute of Standards and Technology*; V. Silin,* Georgetown University **INVITED**

Surface plasmon resonance (SPR) studies provide real-time information about the nature of the interaction of phospholipid vesicles and red blood cell ghosts with alkylthiol modified surfaces. By using a focussed beam, a range of incident angles are detected simultaneously at a linear CCD array, and the reflectivity response is evaluated at each time point. With this approach it is possible to observe time-dependent changes in the width of the reflectivity response as well as in the angular dependence of the reflectivity minimum. The width of the reflectivity response is an indicator of surface roughness, and helps to elucidate the details of the surface reaction. At a hydrophobic surface, vesicles disassemble and add a monolayer of lipid to the surface, forming a smooth hybrid bilayer that is a model of a cell membrane. This process occurs with vesicles that are net neutral or carry a net negative charge. The interaction of red blood cell ghosts with the surface results in increases and then decreases in the width of the reflectivity response, presumably as a result of initial binding of the cell membrane particles, and then reorganization of the cell membrane into a discrete layer at the hydrophobic surface. The interaction of vesicles with a charged surface is distinctly different from their interaction at a hydrophobic surface. Instead of addition of a layer of lipid, vesicles apparently remain intact at the charged surface, resulting in a large shift in the angle of minimum reflectivity, and an increased and sustained broadening of the reflectivity response. This broadening reflects heterogeneity in the surface coverage, suggesting the long-term presence of intact vesicles at the surface.

9:40am **BI-TuM5 Stabilizing Supported Lipid Bilayers for Biomaterial Applications**, ***O. Dannenberger***, *M. Boeckl, J.A. Bassuk,* University of Washington*; P.L. Valint,* Bausch & Lomb*; T. Sasaki, V. Vogel,* University of Washington

Supported phospholipid bilayers are promising biomembrane model systems.@footnote 1@ Our special interest is to extend their application to investigate the interactions of cells with specific recognitions sites on synthetic surfaces. Bilayers are ideally suited to stabilize and expose cell receptors and membrane anchored proteins. The fluid character of supported bilayers further allows cells to spatially reorganize recognitions sites. As one major drawback of lipid bilayers is their instability we have assembled phospholid (PL) bilayers on a smooth hydrogel cushion and partially crosslinked the inner layer to the cushion and the layers themselves. The hydrogel mimics the cytoskeletal network that stabilizes the lipid membranes in living cells. HEMA was selected as the major polymer constituent due to its biocompatibility. The PL bilayers were formed using Langmuir Blodgett and Schäfer techniques. The hydrogels were investigated with AFM and angle-resolved XPS, the mono- and bilayers were studied with fluorescence and Brewster angle microscopy. We covalently linked our inner layer to the reactive groups provided on the polymer cushion. While partial linkage stabilized the inner leaflet it still exhibits a high membrane fluidity since direct interactions with the solid substrate are prevented. Bifunctional lipid-like surfactants were used to improve the stability of the outer PL leaflet through layer-to-layer crosslinking. In addition we will present the results of the attachment and spreading behavior of endothelial cells on our model surfaces. @FootnoteText@ @footnote 1@E. Sackmann, Science 271 (1996), 43-48.

10:00am **BI-TuM6 Patterning Multiple Antibodies onto a Surface**, ***R.A. Brizzolara***, NSWC, Carderock Division

Patterning multiple antibodies (each to a different antigen) on a single substrate is an important step in the development of multi-analyte biosensors. The envisioned device requires patterning antibodies onto a substrate in discrete pixels, with adjacent pixels containing antibodies against different antigens. In this paper, a new method for forming patterns of different antibodies on a surface is described. The method utilizes hydrophobic interactions to immobilize antibodies on a polystyrene surface. Prior to incubation in the antibody, the substrate is coated with bovine serum albumin (BSA) to prevent nonspecific adsorption. The BSA is then selectively removed from the region where antibody adsorption is desired. Results will be presented demonstrating several methods of selective BSA removal, including ion-beam sputtering and mechanical scribing. Ion beam sputtering has been used to form a rudimentary, millimeter-scale pattern of two antibodies on a polystyrene surface. X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) were used to detect the spatial distribution of 10 nm gold labeled antigen on the surface. Mechanical scribing has been used to form a 0.1 mm-scale pattern of two antibodies on a surface. Fluorescence Microscopy was used to visualize the FITC labeled antigen on the surface. Finally, results will be presented showing the use of the AFM as a lithographic tool for antibody patterning. The NSWC, Carderock Division In-House Laboratory Independent Research Program provided funding support for this work.

10:20am **BI-TuM7 Biological Applications of Colloidal Au-Amplified Surface Plasmon Resonance**, ***M.J. Natan***, The Pennsylvania State University **INVITED**

The ability to rationally control surface topography of thin noble metal films has an enormous impact on the utility of such films for biosensor signal transduction. Nowhere is this more evident than in surface plasmon resonance (SPR), where changes in surface roughness of a few nanometers are easily detected. Accordingly, when immobilization of colloidal Au nanoparticles from solution is brought about by a biomolecular recognition event on an SPR substrate, dramatic changes in reflectivity are observed. This talk will focus on fundamental aspects of this phenomenon, including the dependence on colloidal Au particle size, particle coverage, and particle spacing, as well as on applications to ultrasensitive detection of proteins, nucleic acids, and small molecules.

11:00am **BI-TuM9 Structure of Single- and Double-Stranded DNA Monolayers on Gold from Neutron Reflectivity**, *R. Levicky, T.M. Herne,* ***M.J. Tarlov***, *S.K. Satija,* National Institute of Standards and Technology, US

Neutron reflectivity was used to determine the concentration profiles of oligomeric DNA monolayers on gold under conditions of high salt (1M NaCl). These monolayers are of interest as model DNA probe systems used in diagnostic devices. To facilitate its attachment, the DNA was functionalized at the 5' end with a thiol group connected to the oligonucleotide by a hexamethylene linker. Concentration profiles determined from neutron reflectivity indicate that adsorbed layers of single-stranded DNA (HS-ssDNA) on bare gold are compact, suggesting the presence of multiple contacts between a DNA strand and the surface. After treatment with mercaptohexanol, a short alkanethiol with a terminal hydroxy group, the DNA "stands-up" and extends farther into the solvent phase. These changes are consistent with the DNA remaining attached through its thiol endgroup while contacts between DNA backbones and the surface are limited by the formation of a mercaptohexanol monolayer. The end-tethered HS-ssDNA layer readily hybridizes to its complementary sequence, resulting in DNA helices with a preferred orientation toward the substrate normal.

11:20am **BI-TuM10 XPS Analysis of Oligonucleotides for DNA Microarrays**, ***E.C. Carr***, *K.J. Luebke, S.M. Lefkowitz, N.M. Sampas, S.S. Laderman, E. Poon,* Hewlett-Packard

In recent years, a vast amount of DNA sequence information has been provided by the Human Genome Project. To make intelligent and efficient use of this information requires new analytical techniques capable of massively parallel interrogation. Microarrays of DNA have emerged as one of the most promising molecular recognition technologies for high sensitivity, multiplex analysis of DNA. It has been demonstrated that specific sequences of DNA can be synthesized directly on a planar surface, however the density and fidelity of the resulting oligonucleotides are of primary importance to the effectiveness of the array. We have used X-ray Photoelectron Spectroscopy (XPS) to measure phosphorus signal, and thus nucleotide density, of oligonucleotides synthesized on a silylated glass surface. We derive a coupling yield for the attachment of each nucleotide in the sequence that is in good agreement with yield derived from optical transmittance of dye bound to the final nucleotide. Coupling yield measured by these techniques is consistent with that achieved in standard DNA synthesis on porous glass beads using cleavable linkage to the bead and High-Performance Liquid Chromatography for measurement. Issues associated with the accuracy of making quantitative measurements on microarrays using XPS will be discussed.

11:40am **BI-TuM11 Multifunctional Molecular Multilayer Assemblies for Reagentless, Fluorescence-Based Biosensing**, *M.M.A. Sekar, P.D. Hampton,* ***G.P. Lopez****,* University of New Mexico

This report describes the development of self-assembled monolayers (SAMs) and surface modified SAMs to create model, multifunctional organic surfaces for reagentless, fluorescence-based biosensing. We describe the formation of modular molecular assemblies that display biospecific ligands, luminescent probes and highly-hydrated oligomeric species that inhibit nonspecific adsorption of biomolecules and cells [e.g., oligo(ethylene glycol)]. SAMs can be formed by the serial chemisorption of amine-terminated alkylthiolates to gold or alkylsilanes to silica, and can be serially modified by reaction with di-aldehydes, and subsequently di-amines, to form layered molecular assemblies that can be terminated with bioreceptive ligands, luminescent probes or oligo(ethylene glycol). This talk will present a detailed surface analytical characterization of these layered molecular assemblies and will discuss the efficacy of this method for constructing modular, multifunctional assemblies for interfacial modification of biomaterials. We demonstrate that protein-sensitive luminescent probes immobilized on such layered assemblies can be used to detect specific and nonspecific adsorption of proteins through steady-state and time-resolved fluorescence spectroscopy.

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