Biomaterial Interfaces Group
Room 326 - Session BI-MoM

Protein Solid-Surface Interactions

Moderator: J.J. Hickman, The George Washington University

8:20am **BI-MoM1 Effect of Surface Rheology on Anti-Adhesive Properties of Water-Soluble Thin Films**, ***J.W. Schneider***, *W.R. Barger, J.-B.D. Green, R.F. Brady, Jr., G.U. Lee,* Naval Research Laboratory

Thin films of water-soluble polymers, including poly (ethylene oxide), have been used to improve the biofouling resistance and biocompatibility of surfaces. Their effectiveness has been traced to strong associations with water, leading to short-ranged, repulsive hydration forces, and to steric stabilization forces, which are related to the restriction of surface mobility as potential adherends approach them. With the rational molecular design of anti-bioadhesive coatings in mind, we work to decouple each of these effects by making sensitive nano-scale force measurements on organized monolayers hosting oligomers of controlled molecular weight and surface density. In this work, we employ the atomic force microscope (AFM) in two modes to obtain these two surface characteristics. Surface rheological properties, which are a measure of the propensity for steric stabilization, are obtained by driving the AFM tip across a frequency spectrum and measuring the phase and amplitude of the cantilever response as a function of probe/surface separation distance. Short-range forces are measured by operating the AFM in conventional static force modulation mode. AFM tips are functionalized to reflect pertinent biological chemistry using thiol SAM's terminated by carboxylic acids, amine groups, and the monosaccharide sialic acid.

8:40am **BI-MoM2 Probing the Local Interaction Forces with the AFM between Tertraglyme and Fluorinated Polymers and Biomolecules**, ***R. Luginbühl***, *Y.V. Pan, B.D. Ratner,* University of Washington

The interactions between biomolecules and surfaces play a major role in biological process. In biomaterial science, a key to success lays in understanding and controlling the local characteristics of the material surfaces. Engineering of recognition surfaces with well-defined chemical and physical properties is of highest interest for fabrication of biocompatible surfaces, biosensor technology, medical devices and/or molecular electronics. Polymer films, deposited in a radio frequency plasma enhanced chemical vapor deposition (RF-PECVD) process, are of increasing interest as biomaterial coatings. Plasma polymerization of tetraglyme (CH3-(O-CH2-CH2)4-O-CH3) was reported by Lopez and Ratner in 1992. The resulting thin films show resistance to protein adhesion, cell and bacteria attachment, similar to poly-(ethylene glycol) (PEG). In contrary to tetraglyme coatings, fluorine-containing films polymerized from perfluorinated monomers are very hydrophobic and exhibit a high interaction to biomolecules and cells. The interactions between biomolecules and a material surface can be probe with the atomic force microscope (AFM) at the molecular level. AFM cantilever tips were modified with a thin film of either one of the above mentioned plasma polymers and/or with biomolecules. The adhesion force was quantified by the mean of force versus displacement curves. In addition, recognition images of polymer patterned surfaces with biomolecule modified AFM tips were obtained applying a new modulation technique.

9:00am **BI-MoM3 Proteins at the Solid-Solution Interface: Significance, Behavior and Manipulation**, ***J.L. Brash***, McMaster University, Canada **INVITED**

Proteins are large, amphiphilic molecules and as such are highly surface-active: they seek out the interfacial environment. Proteins will thus tend to accumulate at any gas-liquid or solid-liquid interface, and in doing so their biological activity is often altered. Such behavior has consequences for many areas of science and technology where protein-containing fluids are present. Examples are protein separation and purification, the "biofouling" of surfaces in bioprocess equipment and biosensors, and the biocompatibility of medical devices such as vascular prostheses and contact lenses. In the case of biocompatibility, the adsorbed proteins determine subsequent cell interactions. A theme which emerges from these considerations is the need to be able to "control" or "direct" protein adsorption. In this presentation the interfacial behavior of proteins will first be discussed. Approaches to the control of protein adsorption will then be suggested. Such control has a number of aspects, including selecting a given protein from a multiprotein fluid (eg blood), controlling the conformation (and thus the function) of adsorbed proteins, and preventing adsorption altogether. Examples from the author's research in the blood compatibility area will be presented. These will include fibrinolytic (clot-dissolving) surfaces based on the preferential adsorption of plasminogen, anticoagulant surfaces based on thrombin scavenging, and protein repellent surfaces based on polyethylene oxide grafting.

9:40am **BI-MoM5 Molecular Recognition Between Genetically-Engineered Streptavidin and Surface-Bound Biotin**, ***V.H. Perez-Luna***, *K.A. Opperman, P.D. Hampton, M.J. O'Brien,* University of New Mexico*; L. Klumb, P. Stayton,* University of Washington*; G.P. Lopez,* University of New Mexico

There are fundamental differences between molecular recognition at the solid-liquid interface and in solution. In solution, ligands and receptors are randomly distributed in space, have high mobility, random orientation and, after binding, the ligand receptor pair can freely move in the solution. At the solid-liquid interface, the immobilized species is concentrated at the solid surface, has low mobility, preferential orientation for the ligand and, upon binding, the ligand-receptor pair becomes constrained to the surface region. Interactions between the immobilized biomolecules and the surface may occur or, at high densities of the immobilized receptors, attractive interactions among adjacent adsorbed ligands can give rise to cooperative effects. In this work, we study such differences with the streptavidin-biotin molecular recognition system. Binding of streptavidin to biotin-terminated self assembled monolayers (SAMs) on gold is reported. Three streptavidin mutants were used in this work: wild type, Y43A and W120A. Desorption of the bound protein molecules was obtained by incubation of the SAMs in 1 mM biotin. Desorption from disordered monolayers was incomplete, which suggests that non-specific interactions occurred either with the gold substrate or hydrophobic moieties of the thiolate after binding. Non-specific interactions did not occur on well organized monolayers and complete dissociation was achieved. Desorption of the surface bound molecules was modeled considering that the bound proteins could come off the surface either by sequential dissociation of biotin-streptavidin bonds or by simultaneous dissociation of two biotin-streptavidin bonds. The calculated dissociation constants differed by several orders of magnitude for the three mutants and they depended on the degree of coverage of surface bound biotin. The later indicating the presence of attractive interactions among adsorbed molecules at high surface coverage.

10:00am **BI-MoM6 Interfacial Influences on the Apparent Activity of Immobilized Electron Transfer Proteins**, ***D.E. Leckband***, *C. Yeung, N. Lavrik, A. Kloss,* University of Illinois, Urbana-Champaign

We determined the influence of the interfacial microenvironment on the apparent activity of immobilized proteins. In particular, we investigated the effect of the electrostatic potential of the underlying support on the interaction of soluble cytochrome b5 with immobilized cytochrome c. By varying solution pH, we controlled the magnitude of the negative charge on the supporting matrix. Because cyt b5 is also negatively charged at neutral pH, the substrate repels the soluble cyt b5 and thus opposes the cyt c/cyt b5 attraction. We show, using surface plasmon resonance, that the apparent pH-dependence of the interprotein affinity is determined largely by the pH-dependence of the substrate, and not by the intrinsic interactions between the two proteins. On the matrix used in this work, we showed that the pH-optimum for the cyt c/cyt b5 recognition shifts by 1.2 pH units relative to that of the soluble proteins. Our results demonstrate that the apparent biological activity of immobilized species must be considered within the context of the microenvironment in which they function.

10:20am **BI-MoM7 Interfacial Supra-Biomolecular Assemblies on Solid Supports**, ***W. Knoll***, The Institute of Physical and Chemical Research (RIKEN), Japan, Germany*; A. Offenhaeusser,* Max-Planck-Institut für Polymerforschung, Germany **INVITED**

This contribution summarizes some of our efforts in designing, preparing, and characterizing supramolecular interfacial assemblies integrating biomolecular functional units. Among the presented bio-interfaces are oligonucleotide matrices fabricated by self-assembly strategies based on thiol coupling to Au-substrates or on biotin-streptavidin interactions. A multispot parallel read-out of hybridization reactions between various surface-bound capture probes and complement strands from solution is presented. The concept is based on surface plasmon microscopy and image analysis computer routines. It is shown how the interfacial architecture can be optimized for maximum binding efficiency by using monomolecular layers assembled from binary thiol solutions composed of the catcher probes and diluent molecules that control the lateral separation of the individual binding sites. First results on the influence of the ionic strength,

the degree of mismatch, and the temperature are presented. In addition the extension of this concept to PNA catcher probes in discussed. The second class of surface architectures concerns tethered membranes. Various concepts for the coupling of lipid bilayers to solid supports of different materials (Au, SiOx) based on polymers or peptides are briefly discussed. Particular emphasis is put on the structural analysis of the complex multilayer assembly and on the functional characterization by electrochemical techniques. The reconstitution of membrane-integral ion translocating proteins into the supported bilayers bears great potential for biosensor formats.

11:00am **BI-MoM9 Qualitative and Quantitative Mass Spectrometric Methods for Probing Surface-Protein Binding Affinity**, ***G.R. Kinsel***, *A.K. Walker, L. Chen, K.D. Nelson, Y. Wu,* University of Texas, Arlington*; R.B. Timmons,* University of Texas, Arlington, U. S. A.

We have recently shown that Matrix Assisted Laser Desorption Ionization (MALDI) Mass Spectrometry (MS) can be used as an efficient tool for characterizing surface protein interactions. When using a standard sample preparation methodology there is a direct inverse correlation between the surface-protein binding affinity and the magnitude of the protein MALDI MS ion signal. The MALDI MS method offers numerous advantages over conventional bioanalytical methods for assaying surface-protein binding including: 1) surface binding of a broad spectrum of proteins and biomolecules can be assayed, 2) no modification (e.g. radiolabeling, fluorescent labeling) of the protein is required, 3) analysis is straightforward and can be completed in under 30 minutes 4) sensitivities are comparable to radiolabeled protein binding studies. Our present research is focused on assessment of the conditions under which the MALDI ion signal can be quantitatively correlated to the surface-protein binding affinity. Specifically we have examined the impact of changes in a variety of surface (morphology, chemical functionality, etc) and protein solution (protein choice, solution pH, etc.) characteristics on the general quantitative MALDI ion signal / binding affinity correlation. These studies reveal both the range of conditions across which the MALDI MS methodology can be applied to quantitate surface-protein binding as well as insights into the relative impact of various surface / solution parameters on the adsorption process.

11:20am **BI-MoM10 Biosensing Using Colloidal Au Arrays as Biocompatible Substrates and Au:Protein Conjugates as Signal Enhancing Agents**, ***M.D. Musick***, *L.A. Lyon, G.P. Goodrich, M.J. Natan,* Pennsylvania State University

Sensing strategies are discussed using arrays of colloidal Au as a sensor substrate and solutions of protein:Au colloid conjugates as signal amplification reagents. Colloidal Au shares similar properties with bulk Au, a common transducer substrate due to its reflectivity, conductivity, and ease of chemical modification. However, colloidal Au offers increased biocompatibility and flexibility. Particles can be assembled directly from solution onto a wide range of supports. Furthermore, particle size and spacing are easily controlled. 2-D and 3-D arrays have been fabricated from combinations of biomolecules, ligands, organic crosslinkers, and colloidal Au. Assemblies have been characterized by AFM, FE-SEM, uv-vis/NIR, electrical resistance, and electrochemical analysis. The stability and immobilization of protein:Au colloid complexes have been examined. An amplified surface plasmon resonance (SPR) sandwich assay is presented. In this assay, a protein layer immobilized on an evaporated Au film is exposed to analyte solution and incubated with a protein:Au conjugate. The result is a enhanced shift in the SPR curve as compared to conventional SPR. The use of colloidal Au amplified surface plasmon resonance should offer increased molecular weight sensitivity and lower detection limits. Imaging and arraying methods that allow for simultaneous analysis of many samples, and sensors based on changes in electrical and electrochemical signals will also be described.

11:40am **BI-MoM11 Electron Transfer of Cytochrome c on Lipid-Coated Graphite Electrode**, ***S. Boussaad***, *R. Arechabaleta, N.J. Tao,* Florida International University

The structural and electron transfer properties of Cytochrome c (Cyt c) Langmuir-Blodgett (LB) films, and Cyt c on Cardiolipin (CL) and Phosphatidylcholine (PC) monolayers have been studied on graphite electrode with tapping mode atomic force microscopy (AFM) and cyclic voltammetry. The protein in the LB film forms an ordered structure and exhibits a reversible electron transfer reaction in phosphate buffer. The analysis of the AFM images reveals a quasi-hexagonal structure with a=4.4 ± 0.2 nm, b=5.3 ± 0.2 nm and @gamma@=71 ± 3°. These dimensions are in good agreement with the X-ray data. The redox peaks of the Cyt c monolayer are about 80 mV more positive than those of the spontaneously adsorbed protein, and the electron transfer rate (20-30 s@super -1@) is smaller than 60-80 s@super -1@, the value for the adsorbed Cyt c. Furthermore, both monolayers of CL and PC are ordered on graphite, but their interactions with Cyt c are quite different. On CL monolayer, Cyt c adsorbs spontaneously and the adsorbed protein preserves the electron transfer reaction. In addition, the protein disrupts seriously the ordered structure of the lipid monolayer. However, on PC monolayer, Cyt c does not adsorb. This difference is consistent with the fact the CL plays an important role in the activity of Cyt c oxidase than PC.

Biomaterial Interfaces Group
Room 326 - Session BI-MoA

Cell Solid-Surface Interactions

Moderator: D.E. Leckband, University of Illinois, Urbana-Champaign

2:00pm **BI-MoA1 Cell Solid-Surface Interactions Under Flow**, ***L.V. McIntire,*** Rice University **INVITED**

Understanding the molecular mechanisms of cell-solid surface interactions is crucial for developing specific therapeutic strategies to control or modulate processes such as thrombosis, inflammation and cancer metastasis. In the vasculature or in blood contacting artificial devices these interactions occur under dynamic flow conditions. Primary adhesion or tethering of the flowing cells requires very special properties of the cell surface receptor and its ligand on the solid structure because of the very short contact times available for bond formation and the forces on those bonds due to fluid drag. Leukocytes utilize primarily members of the selectin family of receptors and their carbohydrate ligands for this first step - often resulting in a rolling interaction. For lymphocytes and monocytes, the integrin @alpha@@sub 2@ @beta@@sub 1@ is also capable of mediating primary adhesion under some flow conditions via its ligand vascular cell adhesion molecule (VCAM). Platelets utilize a receptor complex GPIb-IX-V for this tethering process. Secondary or firm adhesion is mediated by members of the integrin family on the cell surface, often after activation, in concert with their ligands on the solid. For leukocytes these are members of the @beta@@sub 2@ integrin family, while for platelets they are members of the the @beta@@sub 3@ integrin family. Methods for dissecting the specific molecular pathways involved for each step in the adhesion process for several cell types are given and the current state of our knowledge and potential applications are discussed.

2:40pm **BI-MoA3 Leukocyte Adhesion on Self-Assembled Thiol Monolayers under Flow**, ***V.A. Tegoulia,*** *S.L. Cooper,* University of Delaware

The effect of specific chemical functionalities on the attachment of neutrophils, one of the cells responsible for host response to biomaterials, was investigated using self-assembled monolayers (SAMs) containing different terminal groups. A surface modification was used to incorporate a zwitterionic phosphorylcholine group on one of the surfaces. Adhesion was studied on surfaces preadsorbed with fibrinogen or albumin, under well defined flow conditions using a radial flow chamber and automated videomicroscopy. The general formula for the thiols used was HS-(CH@sub 2@)@sub 10@-X, where X=CH@sub 3@, CH@sub 2@OH, COOH, COOCH@sub 3@ and CH@sub 2@(OCH@sub 2@CH@sub2@)@sub 3@OH (EG@sub 3@). The phosphorylcholinated monolayer (PC, X=CH@sub 2@PO@sub 4@CH@sub 2@CH@sub 2@N(CH@sub 3@)@sub 3@ was prepared by phosphorylation of the hydroxyl terminated SAM. Contact angle measurements, ellipsometry and X-ray photoelectron spectroscopy (XPS) were used to characterize the SAMs. The amount of adsorbed protein on the surfaces was quantified using radiolabelled fibrinogen and albumin. Neutrophils were isolated from fresh human whole blood. Contact angle measurements, ellipsometry and XPS confirmed the presence of the SAMs. Phosphorous and nitrogen were detected on the phosphonated SAM. Neutrophil attachment was found to be higher at the low shear rates. Cell adhesion was increased on the hydrophobic CH@sub 3@ and the anionic COOH terminated surface. Cells were more activated on the COOH surface. The presence of the EG@sub 3@ and the PC moieties led to very low cell adhesion.

3:00pm **BI-MoA4 Smart Polymers for Bacterial Release**, ***L.K. Ista,*** *V.H. Pérez-Luna, G.P. López,* University of New Mexico

Poly (N-isopropylacrylamide) (PNIPAAM) was used as a model system to demonstrate the utility of environmentally responsive, or "smart", polymers as agents for the release of bacterial biofilms. PNIPAAM was grafted onto the surface of polystyrene coupons by plasma-initiated in situ polymerization. The resultant grafted polymer exhibited the characteristic lower critical solubility temperature (LCST) of 32 @super o@C, as demonstrated by a change in water contact angle and was characterized by x-ray photoelectron spectroscopy. The surfaces were challenged with bacterial strains of marine (Halomonas marina) and medical (Staphylococcus epidermidis) importance as well as with natural sea water. Under experimental conditions, cells attached at temperatures above the LCST of PNIPAAM were released upon transfer to flow conditions below the LCST. The total release observed was greater than 90% of the initially attached cells. In addition, fouling and release could be repeated on the same sample several times, with a small loss in release efficiency upon each repetition.

3:20pm **BI-MoA5 Characterization of Biorecognition Surfaces**, ***B.D. Ratner,*** University of Washington **INVITED**

Biomaterials can now be surface-engineered to drive and control specific bioreactions in vivo and in vitro. Three examples will be presented: (1) surface-immobilized amino acids, peptides and proteins; (2) templates for protein recognition; and (3) surfaces that resist the deposition of biological materials and therefore act in a "stealth" fashion. These surfaces bring new challenges to surface analysis to deal with the molecular complexity, molecular orientation and 2D and 3D organization found on recognition surfaces. Static TOF-SIMS, XPS, IRAS, and AFM are allowing us to glean new information on such surfaces. This overview will highlight progress made in analysis of complex recognition surfaces and demonstrate relationships between surface structure and biological response.

4:00pm **BI-MoA7 Neuronal Networks as the Basis for Computational Systems**, ***J.J. Hickman,*** *M.S. Ravenscroft,* The George Washington University

We are using patterned Self-Assembled Monolayers (SAMs) to control the intrinsic and geometric properties of cell culture growth surfaces to create in vitro circuits of mammalian neurons and their processes. The ability to control the surface composition as well as other variables, such as growth media and cell preparation, all play important roles in neuronal pattern viability and cell fate. The use of serum-free medium makes examination of the culture substratum possible by surface analysis as the serum-free medium contains very small amounts of protein, thus the protein on the surface arises primarily from the cells and can be related to their morphology. The surfaces have been characterized by X-ray Photoelectron Spectroscopy (XPS) and imaging XPS using a FISONS 220i spectrometer and we have related the intrinsic properties of the SAM surfaces and the deposited protein layer to the neuronal cellular development. The electrophysiological signals produced by the neurons in response to artificial and spontaneous electrical stimuli has been recorded by patch-clamp electrophysiology. We are using these circuits to obtain a more fundamental understanding of neuronal circuit development as well as to develop new concepts of hybrid neuroelectric devices for biological computation applications. The continuing development of this technology by our group and other groups will be discussed, as well as the application of this technology for (a) obtaining an improved understanding of neuronal synaptic development, (b) formation of neuronal circuits, and (c) biosensor fabrication. The theory behind the creation of simple hybrid devices will also be explored.

4:20pm **BI-MoA8 Growth of Central Nervous System Cells on Microfabricated Pillars**, ***A.M. Perez,*** *S.W. Turner,* Cornell University*; N. Dowell,* New York State Department of Health*; L. Kam,* Rensselaer Polytechnic Institute*; J.N. Turner, W. Shain,* New York State Department of Health*; R.C. Davis, M. Isaacson, H.G. Craighead,* Cornell University

We are investigating the influence of microfabricated micrometer-size surface features on the attachment and growth of mammalian central nervous system cells. Columnar surface structures have been fabricated using photolithography and reactive ion etching to create arrays with varying sizes and separations. Features 1 µm in height and 1.0 - 5.0 µm in diameter separated by 0.5 - 4.0 µm have been patterened on silicon wafers. The patterned wafers possess 50 µm wide regions of pillars surrounded by smooth silicon surfaces. Several pillared surfaces were also chemically modified with biological polymers including polylysine and conjugated laminin to study the behavior of cells on chemically treated topography. Cells used for these studies include LRM55 astroglial cells, cortical astrocytes prepared from primary cultures, and hippocampal neurons. Cell growth was characterized by scanning electron and fluorescence microscopy while focal contacts and cytoskeletal elements were determined using techniques of vinculin immunocytochemistry and actin cytochemistry, respectively. Astroglial cells preferentially attached to the pillars as opposed to the smooth surrounding surfaces while neurons attached randomly. Cell densities both on and off the pillars have been measured using optical microscopy. The cell densities and morphologies varied according to the geometric features of the columnar surfaces.

4:40pm **BI-MoA9 Directed Neuron Attachment and Growth by Micrometer-Scale Chemical Patterning of Glass Substrates**, ***C.D. James,*** *R.C. Davis,* Cornell University*; L. Kam,* Rensselaer Polytechnic Institute*; H.G. Craighead, M. Isaacson,* Cornell University*; J.N. Turner, W. Shain,* New York State Department of Health, University of Albany*; G. Banker, G. Withers,* Oregon Health Sciences University

Directed neuron attachment and growth is a necessary technology for long-term, in vitro studies of synaptically interactive neurons. Research has shown that chemical cues can stimulate cell attachment and neurite outgrowth in neurons when cultured on chemically-modified bioactive surfaces. Specifically, the synthetic polypeptide polylysine has been shown to induce cell attachment, and the basement membrane protein laminin has been used to initiate neuronal process outgrowth as well as cell attachment. In this paper, we attempt to produce a method for using chemical cues to control the organization of neurons into defined networks in order to facilitate long-term studies of synaptic function and inter/intra-neuronal signal processing. We demonstrate a technique for chemically patterning glass substrates with polylysine and laminin using microcontact printing, an emerging tool for micrometer-scale chemical patterning of surfaces. Further, we show that these chemically patterned surfaces are biologically active, and that cell attachment and neurite outgrowth are stimulated in culture.

5:00pm **BI-MoA10 Characterization of Cellular Interfacial Forces with AFM**, ***T.J. Boland,*** Pennsylvania State University*; Y.F. Dufrene,* Universite Catholique de Louvain, Belgium*; W.R. Barger,* Naval Research Laboratory*; D.L. Allara,* Pennsylvania State University*; G.U. Lee,* Naval Research Laboratory

Biomaterial design depends on understanding the molecular basis of material-body interactions. Much is known about the molecules and cells involved in the body's response to foreign materials but it has been difficult to characterize the physical nature of their interaction. To this end, the interfacial properties of model films have been measured at the nanometer scale with atomic force microscopy (AFM). In specific, as a model for cell surfaces, mixed, uncharged phospholipid/glycolipid monolayers have been deposited on octadecyltrichlorosilane monolayers (OTS) using Langmuir-Blodgett (LB) deposition. The lipid films phase segregate allowing us to measure the relative surface properties of the different phases. Spectroscopic ellipsometry was used to characterize optical properties and thickness of each pure layer and the mixed bilayers in air and in water. As a model for a polymeric surface, AFM probes were functionalized with SH-(CH@sub 2@)@sub 15@-R, where R=CH@sub 3@, CH@sub 2@OH, COOH groups. The height, friction, mechanical properties and surface forces of the lipid phases were measured with these probes. The force curves are purely repulsion due to a dominant short-range force indicative of steric/ hydration interaction and the range of this force is dependent on the head group of the lipid. At high loading forces the probe is observed to snap into contact with the surface which we believe is a measure of the mechanical stability of the film. These measurements demonstrate that AFM can be used to directly characterize molecular interactions between model cell surfaces and model organic surfaces.

Biomaterial Interfaces Group
Room Hall A - Session BI-MoP

Biomaterial Interfaces Poster Session

BI-MoP1 Molecular Structure of Protein-Resistant Plasma-Deposited Oligoglyme, Dioxane, and Crown Ether Films, ***E.E. Johnston***, *B.D. Ratner,* University of Washington

Poly(ethylene oxide)-like (PEO-like) films are of interest as biomaterials for their ability to resist protein adsorption and cellular attachment.@footnote 1,2@ Here we compare the chemistry and molecular structure of PEO-like films that were plasma deposited from linear and cyclic ether rich precursors. The plasma precursors consist of a series of four linear oligo ethylene glycol dimethyl ethers (oliglymes) (CH@sub 3@-O-(CH@sub 2@CH@sub 2@O)@sub n@-CH@sub 3@, n = 1-4) and a corresponding series of cyclic oligomers -(CH@sub 2@CH@sub 2@O)@sub m@-: dioxane, (m=2); 12-crown-4 ether, (m=4); and 15-crown-5 ether (m=5). Analysis by x-ray photoelectron spectroscopy (XPS), and time of flight SIMS (TOF-SIMS) will be reported. The view emerging from the analysis is that oligoglyme plasma deposited films consist of a carpet of randomly branched, methyl-terminated chains of -(CH@sub 2@CH@sub 2@O)- repeat units. The films are generally free of hydroxyl groups and hydrocarbonaceous domains. Films from larger molecular weight precursors contain longer fragments of intact monomer suggesting that the films are more loosely crosslinked. Proposed models of the oligoglyme PDF surface structure can account for many aspects of the high mass TOF-SIMS spectra. Interpretation of the crown ether PDF spectra was more difficult than that of the oligoglyme films. On the basis of the (45/43+45) positive ion ratio analysis and adventitious binding of sodium and potassium cations it is concluded that the outermost surface of crown ether films consist predominantly of intact cyclic fragments. TOF-SIMS analysis of dioxane films suggests the presence of hydroxyl groups pendant to an unsaturated hydrocarbonaceous matrix and the presence of ether-carbon bearing precursor fragments. @FootnoteText@ @footnote 1@ L@aa o@pez, G. P.; Ratner, B. D.; Tidwell, C. D.; Haycox, C. L.; Rapoza, R. J.; Horbett, T. A. J. Biomed. Mater. Res. 1991, 26, 415-436. @footnote 2@ Johnston, E. E., PhD dissertation, University of Washington, 1997.

BI-MoP2 Curvature-Induced Domain Formation in Lipid Bilayer Membranes, ***C.D. Keating***, *T.G. D'Onofrio, M.J. Natan, P.S. Weiss,* Pennsylvania State University

A number of studies have suggested the importance of domain formation in biological membranes. We describe recent results concerning the effect of lipid bilayer lateral heterogeneity on model membrane function in single unilamellar vesicles. Initial work has focused on preparation and imaging of lipid membranes of various curvatures, and manipulation of these membranes in flowing solution by optical trapping. Several methods have been applied to alter liposome morphology, from encapsulation of materials (both biological and nonbiological) to stretching vesicles using multiple optical traps. Fluorescence microscopic data on lateral domains as a function of membrane curvature will be discussed.

BI-MoP3 In Situ AFM Study of Myoglobin Monolayers on Bare and Modified Graphite, *M.J. Giz,* IQSC/University of Sao Paulo, Brazil; ***S. Boussaad***, *N.J. Tao,* Florida International University

We have examined with the atomic force microscopy (AFM) technique the structure of myoglobin (Mb) monolayers on graphite. This protein adsorbs weakly and slowly on bare graphite. The formation of a Mb monolayer can take up to 80 minutes. Furthermore, the molecules of Mb form a rod like assemblies randomly distributed on the surface of graphite. The length and the width of a single rod are 50 and 8 nm, respectively. The value of the width is similar to the dimension of a single protein. However, on graphite modified with a monolayer of Didodecyldimethylammonium bromide (DDAB) or Lauric acid (LA), the Mb is well ordered. The AFM images show that the protein preserves the rod-like assembly. In the case of DDAB, the Mb rods are aligned and almost parallel to each other, whereas on LA the rods are arranged into a V-type structure. In addition, the rods formed on modified graphite (70-80 nm) are much longer then their counter part on bare graphite. The Mb rods can be viewed as chains of 5-10 molecules and their formation can be attributed to an interaction between the proteins.

BI-MoP4 Nanofabricated Structures for Laser Induced Fluorescence, ***M.E. Foquet***, *A. Lopez, S.W. Turner, H.G. Craighead,* Cornell University

Fabrication techniques of microfluidic systems are being developed for use in laser induced fluorescence studies of macromolecules. Devices with submicrometer size capillaries have been fabricated on glass substrates for the study of electrophoretic motion of biopolymers. The motion of individual DNA molecules can be observed and their speed estimated. Other devices integrating both optical waveguides and capillaries have been fabricated. The waveguides are included to perform fluorescence using lasers as light source, allowing for the excitation of very small volume combined with a very high intensity and a perfect alignment to the capillary. Gratings defined by electron-beam lithography are used for the coupling of light into the waveguide. The same fabrication process can readily be used to fabricate capillaries with dimensions down to 0.1 µm. Light has been coupled into the waveguide and the patterns of scattered light have been recorded. Excitation of fluorescent solution in the capillaries can be observed. We are now characterizing the efficiency and the optical properties of these structures.

BI-MoP5 Effects of Surface Finish on the Corrosion of NiTi Shape Memory Alloy, ***S. Trigwell***, VG Scientific; *G. Selvaduray,* San Jose State University

NiTi (nitinol) alloys have become very attractive to biomedical applications because of their unique shape memory and superelastic properties. Due to the high Ni content of the alloy (up to 55%), concern has been expressed as to its biocompatibility. Nickel is known to cause toxic reactions in the body and be a possible carcinogen in cases of long term exposure, such as in implants. In this study, coupons of an equiatomic alloy were prepared by four methods (mechanically polishing, electropolishing, chemically etching, and plasma etching) to produce various levels of roughness and surface chemistry, as examined by AFM, SEM, XPS, and AES. The corrosion current (rate) for each finish was determined in Hank's Balanced Salt Solution at 37°C. The correlation between surface finish and corrosion susceptibility was determined that may be important in the preparation of NiTi for biomedical applications.

BI-MoP6 Second-Harmonic Generation in Thin Films of Bacteriorhodopsin: Sensor for Organic Electronic Devices, ***N.V. Didenko***, *A.A. Fedyanin, T.V. Murzina, E.P. Lukashev, O.A. Aktsipetrov,* Moscow State University, Russia

The nonlinear-optical method of second-harmonic generation (SHG) that has been shown to be a sensitive probe for studying surfaces, interfaces and thin films is suggested as a new non-destructive pH-sensor for biological display elements and readout of information for photochromic optical storage. The pH-induced changes of quadratic susceptibility of oriented micron-thick bacteriorhodopsin D85N films obtained for basic titrations (pH range 5-11.5) have led to drastic (more than an order of magnitude) variations in the SHG response. The photo- and electroinduced effects in SHG and Hyper-Rayleigh Scattering (HRS) in solid films of native bacteriorhodopsin are shown to be quite sufficient to distinguish by means of SHG and HRS quazistable states of bacteriorhodopsin molecule which can be used as functional states of memory storage elements. The output of a Q - switched YAG laser at the wavelength of 1064 nm that has been used as a fundamental radiation is shown not to cause the destruction of the samples and switching the memory element as well.

BI-MoP7 Observation of Infected Lymphocyte Cells by Atomic Force Microscopy, ***A. Cricenti***, *R. Generosi, M. Girasole,* Consiglio Nazionale delle Ricerche, Italy; *C. Colizzi, S. Bach,* Universita' di Roma Tor Vergata, Italy; *P. Perfetti,* Consiglio Nazionale delle Ricerche, Italy

The interaction between lymphocyte cells and HIV virus have been studied at the membrane level by Atomic Force Microscopy in the repulsive regime of contact mode. Morphological characteristics of non infected lymphoid cells and HIV infected cells were easily imaged in fixed and dried cell preparations. After HIV exposure we observed a decrease in surface protrusions (loss of microvilli) and the creation of many dips. Some particles, presumably of viral origin (120 - 130 nm size), were also observed in proximity of the cell surface. Similar changes have been observed by AFM on cells exposed to intense electromagnetic field thus indicating that such cells undergo modifications of their morphology upon suffering from an external agent.

BI-MoP8 Enhancing the Sensitivity and Selectivity of the Solid-Phase Immunoassay Using Water Soluble Polymer Coatings, ***M. Natesan***, *S. Metzger,* Geocenters, Inc.; *R.J. Colton, G.U. Lee,* Naval Research Laboratory

Immunoassays have gained acceptance as both a bioanalytical technique and diagnostic tool due to the availability of antibodies from a variety of

species against a wide range of analytes. These assays are often conducted on a solid surface to facilitate separation of the enzymes or radioisotopes that are typically used to amplify the antibody-analyte reaction. The antibody is usually immobilized on a hydrophobic surface by spontaneous adsorption, which is a process driven by molecule-surface interactions and/or conformational changes. The complex nature of this spontaneously adsorbed film can lead to erroneous signal due to the unwanted adsorption of assay constituents or antibody conformational changes. In this presentation we will describe two new schemes for directly immobilizing antibodies at a surface using a monolayer of water-soluble polymer that resists adhesion of proteins and cells. One technique uses direct covalent immobilization of the antibody to the polymer film while the other uses a molecular recognition interaction for assembly. We will describe the physical properties of the polymer films and compare the activity of the polymer immobilized antibodies to spontaneously adsorbed antibodies.

BI-MoP9 Time-of-Flight Cluster Static SIMS, XPS and XRD Studies of Calcium Phosphate Phases, ***C.C. Chusuei***, *D.R. Justes, M.J. VanStipdonk, E.A. Schweikert, D.W. Goodman,* Texas A&M University

Calcium phosphate phase identification is of interest in the modeling of bone growth on metal oxide surfaces. Ostwaltd's step rule suggests that various phases of calcium phosphate will form first prior to that of the principal phase found in bone, hydroxyapatite (HAP). XRD has been shown to be effective in distinguishing between brushite, octacalcium phosphate, amorphous calcium phosphate, alpha-tricalcium phosphate, beta-tricalcium phosphate and HAP. However, this technique is not sensitive to microscopic amounts of material. The effectiveness of ToF cluster SSIMS and XPS methods in the qualitative analysis of standard calcium phosphate powders are compared with XRD. In addition, results of SIMS and XPS studies the solid-liquid uptake of calcium phosphate on UHV prepared metal oxide are presented.

BI-MoP10 Surface Analysis of Anodic Behavior of 316L SS in SRB-Containing Seawater, ***G. Chen***, Constellation Technology Corporation, US*; D.C. White, R.J. Palmer,* University of Tennessee/Oak Ridge National Laboratory*; S.S. Cristy,* Lockheed Martin Energy Systems

Type 316L stainless steel concentric electrodes were exposed to pure cultures of Desulfovibrio desulfuricans, of Vibrio harveyi and the coculture of these bacteria. A 1 microA/cm@super 2@ current was applied for 3 days while the chemostats were closed systems. Subsequently, aerated fresh medium was added. Impedance spectroscopy detected pitting of the coculture-exposed electrodes after addition of fresh medium. X-ray photoelectron spectroscopic analysis after current application and at the end of the experiment revealed that oxygen reduction induced pitting by: (a) formation of a SO@sub 4@@super 2-@, S@sub 2@O@sub 3@@super 2-@ and SO@sub 3@@super 2-@ mixture, (b) oxidation of relatively stable compounds such as Cr(OH)@sub 3@ and, (c) causing sulfides to penetrate the passive film.

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.

Biomaterial Interfaces Group
Room 326 - Session BI+AS+MM+NS+SS-TuA

Nanoscale to Mesocale Biomaterial Structures

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

2:00pm **BI+AS+MM+NS+SS-TuA1 Self-Assembly of a Multidomain Protein: Fibronectin at Lipid Model Interfaces**, ***V. Vogel***, *G. Baneyx,* University of Washington **INVITED**

Fibronectin, an adhesion protein with multiple recognition sites, mediates cell attachment to synthetic and biological surfaces. In solution, fibronectin exists in a globular state where most of its recognition sites are buried in the protein core. Surface adsorption induces conformational changes in the protein that expose many of these sites. Furthermore, it is known that on the surface of cells fibronectin assembles into detergent insoluble fibers, which are considered to be the main functional form of the protein. Fibronectin is hence a prime example of a protein with multiple recognition sites that can be regulated through environmental control. Unfortunately, the molecular pathways of activation and self-assembly are still poorly understood. We have recently found that fibronectin can self-assemble into fibrillar networks at receptor-free phospholipid monolayer interfaces under physiological conditions. This is a crucial observation since the paradigm in biology is that fibril assembly of fibronectin is mediated by membrane-bound receptor molecules. Availability of a simplified model system allows investigation of the molecular pathways by which appropriate surfaces can activate fibronectin and facilitate self-assembly.

2:40pm **BI+AS+MM+NS+SS-TuA3 Nanofabricated Substrates for Probing Single Biomolecules by Surface Enhanced Raman Scattering**, ***S. Petronis***, *L.K. Hedberg, H. Xu, M. Käll, B. Kasemo,* Chalmers Univ. of Technology and Univ. of Gothenborg, Sweden

The effect of Raman scattering enhancement when coherent laser light interacts with molecules attached to rough surfaces and microscopic metal domains has been known for more than two decades and is called Surface Enhanced Raman Scattering (SERS). The intensity of the Raman signals for such molecules is frequently enhanced by a factor 10@super 5@-10@super 6@ at best.@footnote 1,2@ However recently much larger enhancement factors, in the range 10@super 14@-10@super 15@, have been observed for molecules adsorbed on colloidal silver particles of specific dimensions.@footnote 3,4@ This giant enhancement allows the recording of vibrational spectra from a single molecule for the first time, instead of the ensemble averaged spectra from many molecules, which are normally obtained in optical spectroscopies. Here we report on an attempt to use nanolithography to fabricate structures of silver in the size range 100 - 200 nm and having different shapes in order to explore the size and geometry dependence of the SERS effect. Microfabricated structures which give the highest enhancement could be used for probing different biomolecules and perhaps designing a biosensor. SERS active substrates were prepared as arrays of silver particles on a Si wafer. Within each array the silver particles had a constant shape, size and separation. Three particle shapes (circular, triangular and square), two particle sizes (100 nm and 200 nm), and five different particle separations (10, 50, 100, 150 and 200 nm) were produced by electron beam lithography with a double-layer resist system and "lift-off" procedure. A reference area of uniformly deposited Ag film mimicked an infinite silver surface. The final structures and the chemical composition of the silver particles were characterized by Scanning Electron Microscopy (SEM) and Auger electron spectroscopy (AES), respectively. Preliminary Raman scattering experiments have been performed on the dye-molecule Rhodamin 6G adsorbed on the nanofabricated substrates. A giant enhancement of the Raman signal was observed on all patterns, but not on the Ag film or the Si surface. @FootnoteText@ @footnote 1@M.Moskovits, Rev. of Mod. Phys., vol. 57, No 3, 1985, pp 783-826 @footnote 2@A.G.Mal'shukov, Phys. Rep., vol 194, Nos 5&6, 1990, pp 343-349 @footnote 3@K.Kneip et al., Phys. Rev. Lett., vol. 78, No 9, 1997, pp1667-1670 @footnote 4@S.Nie, S.R. Emory, Science, vol. 275, No 21, 1997, pp 1102-1106

3:00pm **BI+AS+MM+NS+SS-TuA4 Nanostructured Surfaces for Biorecognition - A Novel Templating Approach**, ***H. Shi***, *B.D. Ratner,* University of Washington

Materials that specifically recognize proteins may find a variety of applications in separations, sensors and medical materials. Molecular imprinting provides an intriguing approach to plastic antibodies against small molecules, but the use of proteins as templates has been less successful in making protein recognition materials. In this study, nanostructured surfaces with tailored protein-binding cavities are prepared by an imprinting technique based on RF-plasma deposition of organic thin films. A polysaccharide-like surface with protein-imprinted nanopits allows only the template protein to fill the pits, and to bind strongly, because the nanopits are complementary to the template protein in shape and in the distribution of functional groups. The bound protein in its pit is prevented from exchange with protein in the solution due to a strong binding and steric hindrance, while the non-template protein that is weakly adsorbed on the surface is displaceable. Atomic force microscopy (AFM) and transmission electron microscopy (TEM) showed that nanometer-sized pits, in the shape of imprinted proteins, were created on the surfaces of our protein-imprinted polymer films. Imprinting fidelity was confirmed by AFM analysis of imprints of monodisperse colloidal gold nanoparticles. Electron spectroscopy for chemical analysis (ESCA) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) indicated that template proteins were washed off the surfaces of protein imprints while sugar molecules were covalently incorporated. Radiolabeled -protein adsorption showed that a protein imprint recognized its template protein from a binary mixture with a high specificity. This study illustrates a novel templating strategy for biological molecules that can be exploited for fabrication of biorecognition materials.

3:20pm **BI+AS+MM+NS+SS-TuA5 Sensing and Analyzing Single Molecular Interactions with Microfabricated Devices@footnote 1@**, *J.-B.D. Green,* ***G.U. Lee***, Naval Research Laboratory **INVITED**

There is an intense effort to create new tools for manipulating and characterizing single macromolecules because of the power that these techniques can bring to the analysis of biological macromolecules. Due to the high force and displacement sensitivity of the atomic force microscope (AFM) it has been used to measure inter- and intramolecular forces between model ligand-receptors, i.e., streptavidin-biotin, complimentary strands of DNA, and biologically relevant supra-molecular structures, i.e. titin. With the success of these measurements, there are efforts to obtain even more detailed force measurements and to establish these techniques in the biotechnology laboratory. Our efforts focus on: 1. Designing force transducers with force (10@super -12@N), time (10@super -5@s) and spatial (10@super -9@m) resolutions that push the thermal noise envelope. 2. Developing immobilization strategies that produce more reliable force measurements. We will discuss two new microfabricated devices under development in our laboratory. The first microfabricated apparatus offers an excellent platform for detailed measurements of intermolecular interactions and possibly even analysis of combinatorial arrays. The second is an ultra-sensitive detector based on piezoresistive force transduction and magnetic microparticles. The future of these and similar devices will be considered. @FootnoteText@ @footnote 1@This work has been conducted in collaboration with Alexey Novoradovsky, Jonah Harley, Mohan Natesan, Steven Metzger, David Baselt, and Richard Colton.

4:00pm **BI+AS+MM+NS+SS-TuA7 Nanomechanical Properties of Cellular Components Determined by Interfacial Force Microscopy**, ***P.R. Norton***, *K de Jong, J.F. Graham, N.O. Petersen,* University of Western Ontario, Canada

The cell membrane is the contact surface between the cell's internal environment and the outside world. Increasingly it is recognized the there is strong active coupling between mechanical properties and cellular functions in properties such as locomotion and adhesion and in cytoskeletal diseases such as muscular dystrophy.@footnote1@ There is therefore an urgent need to understand the mechanical properties of cells and cellular subcomponents at length scales << 1µm. We will describe our initial experiments to achieve this goal. We have used three different imaging techniques in our investigation of the nanomechanical properties of larynx cells. First, immunofluorescent labelling was used to permit visualization of specific cell components in the confocal microscope, for example to determine whether the cell nucleus was removed in a shearing process. The same cell was then imaged in the atomic force microscope (AFM), permitting identification of components involved in motion such as microspikes. The nanomechanical properties of cells were then studied by nanoindentation using the interfacial force microscope (IFM).@footnote2@ While we have not yet succeeded in imaging and measuring the same cell used in the confocal and atomic force microscopies, we have demonstrated the feasibility of our approach and have obtained quantitative force-distance curves on different regions of a single cell fixed in paraformaldehyde, sodium periodate and lysine, which cross-links the proteins. From these data we can derive the elastic modulus, hardness etc of the specific region of the cell. The modulus of such a cell was ~ 3GPa, comparable to a soft polymer. Similar measurements are planned on unfixed cells. @FootnoteText@

@footnote1@Chen, C.S., et al. Science 276, 1425 (1997) @footnote2@Warren, O.L., et al. Physics in Canada 54, 122 (1998)

4:20pm **BI+AS+MM+NS+SS-TuA8 Unbinding Force of NTA-M@super 2+@--Histidine Complexes. The His-Tag Immobilization Force***, J.G. Forbes, P. Yim,* University of Maryland, College Park

A sequence of six or more histidines will bind tightly to a Cu, Ni, or Co complex. The compound typically used to immobilized the metal is N-(5-amino-1-carboxypentyl)iminodiacetic acid (NTA). Most proteins will not bind to the complex unless there is a sequence of histidines, which is readily added using recombinant DNA techniques. The histidine tag may be removed from the metal complex with a high concentration of imidazole or by protonating the histidines at a pH below 6. We have studied the the unbinding strength of this interaction with the atomic force microscope (AFM). To perform this measurement, we have functionalized silicon nitride AFM tips with NTA-M@super 2+@. A glass slide was coated with recombinant DNAse I with a his-tag on the C-terminus. Unbinding force measurements were made in phosphate buffered saline (PBS) to reduce electrostatic interactions. We find that the unbinding force for the NTA-M@super 2+@/His-tag interaction to be ca. 85~pN for each of the metal complexes. Interestingly, 0.5~M imidazole does not remove the interaction, but only changes the distribution of the measured forces. This is a result of the non-equilibrium condition of the tip being forced into the protein coated surface. The interaction is almost completely removed by lowering the pH to 5.0 where the histidines are protonated and can no longer coordinate with the nickel. The remaining interaction forces are due to the histidines which are exposed when the tip presses into the surface. These results provide a quantitative measurement of mechanical strength of binding of proteins to surfaces functionalized with NTA-M@super 2+@.

4:40pm **BI+AS+MM+NS+SS-TuA9 Sieving of DNA Molecules in Nanofluidic Channel***, J. Han, H.G. Craighead,* Cornell University

Entropic trapping and sieving effect of long DNA molecules was studied in variable thickness nanofluidic channels. We used photolithography and etching techniques to define fluid channels on Si wafers, and anodic bonding method to seal the channel with a thin pyrex glass coverslip. The channel consists of alternating regions with two different channel thicknesses(~100nm and 1.6μm). We studied electrophoretic motion of lambda phage DNA in this channel by epi-fluorescence microscopy. Since the radius of gyration of a typical long DNA molecule is larger than the smaller gap of the channel, the shallow part of the channel can be an entropic barrier for DNA motion.Therefore, DNA molecules were retarded when they entered into the thin region from the thick region. We measured the mobility of DNA molecules in these channels and observed that below a certain electric field, mobility of DNA molecule decreased to near zero drastically, showing that DNA molecules be entropically trapped and sieved. The threshold electric field was mainly dependent on the geometry of channel(e.g. gap size) and the length of DNA driven. This suggests a new type of separation device for DNA and other polymers.

5:00pm **BI+AS+MM+NS+SS-TuA10 Detection of Molecular Ion and Quantification of Pentapeptide on Plasma Hydroxylated Fluoropolymer by Time of Flight Secondary Ion Mass Spectrometry***, J.A. Gardella, L.M. Sun,* State University of New York, Buffalo

Abstract: Poly(hexafluoropropylene-co-tetrafluoroethylene) (FEP) was modified by a hydrogen/methanol radio frequency glow discharge plasma. Time of Flight Secondary Ion Mass Spectrometry (TOF-SIMS) was employed to characterize the modified FEP surface and three pentapetides (YGGFM, YGGFL, YIGSR) which were microsyringe deposited on the modified FEP film. New fragments of OH (CF@sub 2@)n in negative ion SIMS of the modified FEP film indicated that -OH functional group had been incorporated on the FEP surface after plasma treatment. In the positive ion SIMS of three pentapeptides on the hydroxylated FEP film, protonated molecular ions were dominant signals from the peptides whereas not many fragments were observed either from the peptides or the impurity. Sodium and potassium adduct molecular ions were detected as well as oxidized protonated molecular ion of YGGFM in the positive ion SIMS spectrum. Negative ion SIMS of YGGFL yielded a deprotonated molecular ion. The mixture of these three pentapetides was also studied by TOF-SIMS. The relative intensity of protonated molecular ions of YGGFL, YGGFM and YIGSR showed the possibility of quantification on the hydroxylated fluoropolymer by TOF-SIMS. As a study of substrate effects, TOF-SIMS spectra of these peptides on oxidized Ag substrate were recorded. Comparing SIMS results of pentapetides on Ag and on modified FEP film, fewer fragments occurred from the FEP film than that from the Ag substrate. A substrate like the FEP fluoropolymer might be beneficial for the quantification of peptides because of the intensity of parentlike species in SIMS measurement.

Applied Surface Science Division
Room 307 - Session AS+BI+SS-WeM

Organized Molecular Monolayers
Moderator: H.G. Tompkins, Motorola, Inc.

8:20am **AS+BI+SS-WeM1 Structural Characterization of the Outermost Surface Monolayers of CH@sub 3@ and CF@sub 3@ Terminated n-Alkanethiol Monolayers Self-Assembled on Au(111)**, *L. Houssiau,* ***J.W. Rabalais***, University of Houston

Time-of-flight scattering and recoiling spectrometry (TOF-SARS) was used for surface elemental and structural characterization of hexa- and hepta-decanethiols (C@sub 16@ and C@sub 17@ for short) and of 16,16,16-trifluorohexadecanethiol (FC@sub 16@) self-assembled monolayers (SAMs) on a Au(111) surface. The substrate was also characterized after in-situ sputtering and annealing by TOF-SARS and LEED in order to identify its crystalline orientation. The azimuthal angle scans performed on the C@sub 16@ samples displayed unique features, indicating well-ordered structures. The recoiling intensities of the H and C atoms exhibited a clear 60° periodicity, with H recoil maxima and C recoil minima displayed at 30° from the substrate nearest-neighbor directions. Several models were tested by means of classical ion trajectory simulations using the scattering and recoiling imaging code (SARIC). The molecular length, tilt angle, and twist angle were varied in the simulations. Good agreement between the experiments and simulations for both C and H signals was obtained for a (@sr@3x@sr@3)R30 structure of SAM lattice, with the molecules tilted along the substrate nearest-neighbor directions by 35° from the surface normal. Moreover, a twisting of the molecular plane by 55° from the plane normal to the surface had to be considered in order to reproduce the H angular variations. Similar measurements on the C@sub 17@ samples showed much weaker variations for the H and C recoil intensities. This is believed to be due to the high tilt angle of the end methyl group. The FC@sub 16@ samples showed the presence of C and F atoms in the surface layer. No evidence for H and C recoil azimuthal variation was observed on the FC@sub 16@ samples, although a weak azimuthal periodicity was noticed for the F recoils.

8:40am **AS+BI+SS-WeM2 Fundamental Studies of Phase Transitions in Functionalized Amphiphile Monolayers**, ***G.E. Poirier***, National Institute of Standards and Technology

Alkanethiol molecules chemisorb strongly to noble metal surfaces. Intermolecular dispersion forces compress the molecules into a dense, commensurate, crystalline film that spontaneously terminates growth at one molecular monolayer. The thickness of the film, its dielectric constant, and the physical properties of the exposed surface can be controlled by changing the length of the alkyl chain, its degree of saturation, and its terminal functional group, respectively. These systems are therefore desirable in any application requiring control of the adhesive, tribological, or electron transfer properties of metal surfaces. This talk will outline experiments in which methyl-terminated alkanethiol monolayers were prepared by vapor deposition onto clean Au(111) in ultra-high vacuum and characterized in-situ using molecular-resolution scanning tunneling microscopy.

9:00am **AS+BI+SS-WeM3 Characterization of Fluorinated Monolayers**, ***D.G. Castner***, University of Washington **INVITED**

Self assembly processes have made it possible to prepare organic monolayers with well-defined surface structures and chemistries. The ability to systematically vary the surface structure and chemistry of these self-assembled monolayers (SAMs) provide an excellent method for examining the relationship between the surface properties of a film and its performance in a given application (e.g., cell culture). To develop these relationships requires detailed characterization of the surface composition, molecular structure, orientation, and topography of the films with techniques such as XPS, ToF SIMS, NEXAFS, and STM. How the methods and molecules used to form fluorinated films affect the resulting film structure will be discussed. The degree of ordering and relative orientation in SAMs prepared with perfluoroalkyl thiols depend on the length of the fluorinated tail. Although SAMs prepared from thiols with long perfluoroalkyl tails are highly ordered, their stability is limited by the single point attachment of the Au-S bond. This stability can be increased by using grafted copolymers containing both perfluorinated and alkanethiol side chains to generate multipoint attachment of each polymer chain. However, this method of increasing film stability also results in a decreased degree of film orientation. Typically films prepared using polymers with perfluorinated side chains are highly disordered. Only at high concentrations of perfluoroalkyl side chains (>50%) is any ordering detected in polymeric monolayers. Other methods, such as trifluoroacetic anhydride derivatization of hydroxyl-terminated alkanethiols can also be used to produce fluorinated surfaces. Depending on the derivatization system, the high degree of ordering in the starting SAM can be retained after derivatization.

9:40am **AS+BI+SS-WeM5 Controlling Defects in Self-Assembled Monolayers**, ***J.J. Jackiw***, *J.J. Arnold, J.A. Johnson, T.D. Dunbar, T.L. Spiva, D.L. Allara, P.S. Weiss,* The Pennsylvania State University

Much is known about the formation, structure, stability, and properties of alkanethiolate monolayers on Au (111). We have begun to explore the effects of changing the molecule-surface linkage. In the cases of alkanethiolates and alkaneselenolates, monolayers can be made respectively from: thiols and selenols, disulfides and diselenides, and by deprotecting alkanethioacetates and alkaneselenoacetates, which are less prone to oxidation. Our experiments probe monolayer structures and defects resulting from the deposition of dodecanethiol, didodecane disulfide, didodecane diselenide, dodecaneselenol, and in situ deprotected dodecanethioacetate and dodecaneselenoacetate. We compare the structures and defects in the resulting monolayers. The defect identities and densities are important in determining the properties of the films, especially our ability to manipulate their structures and compositions.

10:00am **AS+BI+SS-WeM6 New Preparation Methods for Self Assembly of Alkanethiolates on III-V Semiconductor Surfaces**, ***C.K. Mars***, *D.L. Allara,* Pennsylvania State University

The ability to control the chemical composition of III-V surfaces is vital in semiconductor technology. Alkanethiolate monolayers prepared from thiol melts at temperatures near 100°C have been shown to mimic the improved electronic passivation properties seen on III-V semiconductors with Na@sub 2@S and NH@sub 4@S@sub x@ treatments. We have developed a new process by which these films can be grown reliably in a highly organized state from millimolar ethanol solutions near 50°C. In addition, by adding controlled amounts of base to these solutions, the degree of organization as determined by IR spectroscopy and contact angle measurements, can further be improved. This new process has allowed us to deposit a wide variety of films including aromatic and functionalized molecules as well as layers anchored by other chalcogenide elements.

10:20am **AS+BI+SS-WeM7 The Effect of Solvents and Electrical Fields on the Molecular Conformation in Organic Monolayers**, ***M. Grunze***, *M. Buck, F. Eisert, M. Zolk, P. Harder,* University of Heidelberg, Germany*; A. Pertsin,* Russian Academy of Sciences, Russia*; H.J. Kreuzer,* Dalhousie University, Canada **INVITED**

The molecular conformation in self-assembled monolayers (SAMs) of functionalized alkanethiols is affected by the presence of solvents. The polarity and ability of the solvent molecules to form hydrogen bridge bonds, and the lateral density in the SAMs determines the orientation of the terminal functional moiety at the organic film/solvent interface. We will discuss our in situ Sum Frequency Generation (SFG) and protein adsorption experiments on methyl- and oligo(ethylene glycol)-terminated self-assembled alkanethiolate monolayers, and present atomistic force field calculations to explain our data. These results show that spectroscopic measurements taken in vacuum or ambient atmosphere are not necessarily representative for the molecular structure and chemical nature of organic surfaces immersed in a liquid.

11:00am **AS+BI+SS-WeM9 Nanometer-Scale Design and Fabrication of Polymer Interfaces using Polydiacetylene Monolayers**, ***M.D. Mowery***[1], *M. Cai,* University of Michigan*; H. Menzel,* University of Hannover, Germany*; C.E. Evans,* University of Michigan

The selective control of polymer interfacial characteristics such as viscoelasticity and electronic properties is crucial for numerous important applications from sensor design to device fabrication. In this work, robust interfacial polymer films are successfully fabricated within a single molecular layer with nanometer-scale control of the polymer physical structure. The formation of these unique polymer films is accomplished by the spontaneous assembly of alkyl disulfide precursors containing conjugated diacetylene groups at the gold-solution interface. The resultant well-defined monomer assembly is covalently linked through UV photopolymerization, forming a highly conjugated polymer backbone parallel to the surface. Nanometer-scale control of the interfacial structure is accomplished by manipulating the vertical position of the polymer

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backbone within the single layer assembly. Additionally, photo-templating affords lateral control over the formation of polymer domains. These subtle variations in physical structure have a profound impact on the global electronic and viscoelastic properties of the polymer interface. The impact of these structural variations is demonstrated by surface infrared and Raman spectroscopy as well as electrochemical capacitance, heterogeneous electron transfer, and reductive desorption measurements. Furthermore, AFM is utilized to physically image the interfacial structure and evaluate the polymer viscoelastic properties. Finally, the application of these monolayer polymers for lithographic applications and the implications for interfacial design are discussed.

11:20am **AS+BI+SS-WeM10 Bias-dependent Contrast in STM Images of Phenyloctadecylethers*, I.H. Musselman**, H.S. Lee, S. Iyengar,* University of Texas, Dallas

A homologous series of para-substituted phenyloctadecylethers (X-POEs, X = H, Cl, Br and I) was prepared and characterized using @super 1@H NMR and GC/MS. Scanning tunneling microscopy (STM) images were acquired from monolayers of the ethers physisorbed onto highly oriented pyrolytic graphite. The contrast exhibited by the X-POE molecules in these images varied as a function of tip-sample bias. For example, STM images acquired at biases of approximately -0.5 to -0.6 V (sample negative) exhibited a dim alkyl tail and a series of bright spots corresponding to functionalities in the head group (e.g. halogen, phenyl group, oxygen). However, at biases more negative than -1.2 V, the largest contribution to contrast was observed for the alkyl tail (e.g. octadecyl group). A comparison of STM images of the adsorbed X-POE molecules with electron density contours calculated using HyperChem suggested a bias-dependent participation of individual bonding molecular orbitals to tunneling. A resonance tunneling mechanism between the tip and molecular orbitals adjacent to and including the highest occupied molecular orbital (HOMO) is proposed.@footnote 1@ @FootnoteText@ @footnote 1@ The support of this research by a grant from the Robert A. Welch Foundation is gratefully acknowledged.

11:40am **AS+BI+SS-WeM11 Self-Assembling Trichloro- and Trimethoxy-silanes on TiO@sub 2@(100) Crystal*, R. Magnée**, J.-J. Pireaux,* LISE - Facultés Universitaires Notre-Dame de la Paix, Belgium

Trichlorosilanes (CH@sub 3@(CH@sub 2@)@sub n@SiCl@sub 3@, n = 1, 7, 17) and trimethoxysilanes (CH@sub 3@(CH@sub 2@)@sub n@Si(OCH@sub 3@)@sub 3@ , n = 2, 7, 17) SAM's were deposited by dipping a TiO@sub 2@ (100) crystal in a 10@super -3@M toluene solution for 4 hours. The titanium dioxide surface was prepared by Ar@super +@ sputtering, annealing in UHV to get a clear LEED (1x3) pattern, then exposed to atmosphere before dipping. Silane molecules need H@sub 2@O or a hydroxylated surface to react but it was not necessary to hydroxylate the TiO@sub 2@ because of the air exposure. Hydroxylation was confirmed by FT-IR and XPS. X-Ray and Ultra-violet Photoelectrons Spectroscopy (XPS, UPS) were then used for the SAM's charaterization. We expect a siloxane network to appear at the interface in addition to the Ti-O-Si bonds. XPS results show that the silane groups do indeed bind to the TiO@sub 2@ surface but that some defects are also present at the interface: for n=17 the amount of OH groups is 4 % of the total oxygen signal while this value is doubled for n=7. Probably due to the higher reactivity of chlorine atoms, trichlorosilanes present less defects than trimethoxysilanes. By comparison with theoretical calculations,@footnote 1@ UPS provides information on the alkane chain conformation: we show that longer silane molecules (n=17) are grafted in a zigzag planar conformation, while n=7 molecules seem to present chain defects. This is consistent with the self-assembling process that depends on long-range interchain interactions. For the smallest chains (n=1,2), the XPS C/Si ratios are always too high, suggesting some contamination: this is probably due to solvent incorporation in the layer. HREELS analysis (not yet performed at the date of abstract submission) may help to get more information on the order of the layers. @FootnoteText@ @footnote 1@ A.-S. Duwez, S. Di Paolo, J. Ghijsen, J. Riga, M. Deleuze, J. Delhalle, J. Phys. Chem. B, 101 (1997) 884.

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