Biomaterial Interfaces
Room 307 - Session BI-MoM

Protein-Surface Interactions
Moderator: A. Chilkoti, Duke University

8:20am **BI-MoM1 Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) and Surface Plasmon Resonance (SPR) Determination of Surface Bound Anti-Lysozyme Orientation**, ***N. Xia***, *N.T. Samuel, P.S. Stayton, D.G. Castner,* University of Washington

Static time-of-flight secondary ion mass spectroscopy (ToF-SIMS) and surface plasmon resonance (SPR) were used to analyze the orientation of immobilized proteins. A model protein with a well-define structure, the Fv fragment of a humanized anti-lysozyme (HuLys), was used. A His-tag linked to the C-terminal of the heavy chain was located opposite to the lysozyme binding domain (complementarity-determining region, CDR). We immobilized nitrilotriacetic acid (NTA) onto a self-assembled monolayer (SAM) of oligo(ethylene glycol)-terminated (OEG) thiol on Au. OEG provides a low-fouling background and NTA binds nickel ions, leaving two coordination sites available for interaction with the His-tag of HuLys. SPR experiments showed after nickel activation the NTA/OEG surface specifically bound HuLys Fv via the His-tag and that the immobilized HuLys Fv had nearly full antigen (lysozyme) binding capacity, suggesting a uniform CDR-exposed orientation of HuLys Fv on the surface. For comparison, we also activated the OEG SAM with carbonyldiimidazole (CDI), which binds protein via amine/imidazolyl carbamate chemistry and should result in a random protein orientation. This was supported by the SPR results, which showed that only ~50% of HuLys Fv immobilized on the CDI/OEG surface had antigen binding capability. The ToF-SIMS spectra of HuLys Fv immobilized on NTA/OEG and CDI/OEG surfaces were then compared. It was found that the peaks at m/z=81, 82 and 110 had lower intensities in the spectra of HuLys Fv immobilized on the NTA/OEG surface. These peaks all correspond to the primary mass fragments from the amino acid histidine. Since wild-type Hulys Fv has only 2 histidine residues, the ToF-SIMS results confirmed that HuLys Fv was immobilized onto the NTA/OEG substrate via the His-tag, which should be located at the bottom of the protein layer.

8:40am **BI-MoM2 Identification of Proteins in the Presence of Topographic Features using TOF-SIMS**, ***S. Rangarajan***, *B.J. Tyler,* University of Utah

In an earlier study, we had demonstrated the use of statistical modeling using mixture models and Principal Components Analysis (PCA) in characterizing samples having a relatively simple chemistry and coupled with topographical features. This study involves a very important extension to analysis of samples with more complex chemistry, such as with proteins. ToF-SIMS has been extensively used to characterize proteins, in spite of the inherent difficulties associated with spectral interpretation.@footnote 1,2@ Multivariate methods have also proved to be invaluable in the discrimination of adsorbed proteins on flat substrates.@footnote 3@ In this study, Polystyrene micro-spheres were adsorbed with different proteins and statistical models as well as discriminant techniques such as PCA were used to analyze the ToF-SIMS images. Discrimination of protein spectra from the images was then performed using the afore mentioned techniques, without a priori information about the type of protein adsorbed onto the sphere surfaces. Some of the models, such as the multinomial mixture model were found to yield more information than previously thought. References. @FootnoteText@ @footnote 1@ M.S.Wagner and D. G. Castner, Langmuir 17, 4649 (2001). @footnote 2@ D. S. Mantus and B. D.Ratner, Analytical Chemistry 65, 1431 (1993).@footnote 3@ M.S.Wagner, B.J.Tyler, and D. G. Castner, Analytical Chemistry 74, 1824 (2002).

9:00am **BI-MoM3 Immobilized Microarrays of Capture Agents for Bioassay: A Return to the Past for Protein Surface Stability?**, ***D.W. Grainger***, *P. Gong,* Colorado State University*; M. Lochhead, S. Metzger,* Accelr8 Technology Corporation **INVITED**

Microarrays of antibodies, nucleic acids, and antigens all encounter problems with prolonged bioactivity and desired capture sensitivity in immobilized formats. Surface chemistry is used to produce high target capture activity (high signal sensitivity) with low non-specific binding (noise). These surfaces can exhibit shelf-life problems limited, for example, by intrinsic hydrolysis of amine-reactive coupling chemistry (active esters, aldehydes) even under protective conditions. Reactive commercial array surfaces targeting amine-reactive nucleic acids or proteins have been regenerated in situ using N-hydroxysuccinimide to re-activate amine reactivity, improving functionalization of the commercial surfaces and improving immobilization of amine-terminated probes above original capacity. XPS and ToF-SIMS results for surface re-derivatization are correlated with DNA probe immobilization and target capture efficiencies. In a second effort, contact printed immobilized antibodies against a probe analyte on commercial polymer microarraying surfaces (70-micron spots) were assayed for model target capture (goat IgG) in sandwich immunoassay with fluorescently labeled secondary antibodies in full goat serum, imaged by fluorescence scanning. Off-array noise and on-array signal were compared as a function of printed antibody concentration. Despite masking with prescribed protocols (e.g., BSA or polymer masking), assay signal:noise was markedly improved on a non-masked three-dimensional polymer hydrogel commercial chemistry. Last, commercial arraying surfaces were used to exploit nucleic acid amplification (PCR reaction)on-array, capture fluorescently labeled target amplicons with printed probes, and rinse away all PCR reaction reagents in a single-step assay without prior separations or compromise to signal:noise performance. This provides substantial advantages in time and effort should sufficient signal:noise be achieved without costly, tedious PCR separation steps.

9:40am **BI-MoM5 Limitations of Molecular Streptavidin/Anti-biotin Antibody Architectures using Micro-contact Printed Biotinylated Thiols**, ***Ch. Grunwald***, Ruhr-University Bochum, Germany*; N. Opitz,* Max-Planck Institute for Molecular Physiology, Germany*; S. Herrwerth, W. Eck,* University of Heidelberg, Germany*; J. Kuhlmann,* Max-Planck Institute for Molecular Physiology, Germany*; Ch. Woell,* Ruhr-University of Bochum, Germany

Atomic force microscopy (AFM) and confocal fluorescence microscopy have been used to study the interaction of streptavidin and anti-biotin antibodies with a patterned, biotinylated organic surface. This system presently attracts considerable interest because of its potential for molecular architectures employing protein-protein interactions. The substrates were prepared by first using the µCP technique to print a periodic pattern of an oligoethylenglycol (OEG) self-assembled monolayer (SAM) on clean gold surfaces. The pattern consists of squares (40 µm x 40 µm) which are separated from each other in each direction by 10 µm. By immersing the stamped substrates into a mixture of OH-terminated and biotinylated organothiols a patterned SAM is obtained. These 2D-SAM patterns have been imaged via contact atomic force and lateral force microscopy as well as with tapping-mode AFM. The patterned SAMs were then incubated with two fluorescence-labelled proteins exhibiting a strong affinity towards biotin, streptavidin and anti-biotin antibody. Incubation time, temperature and concentration of the protein solution as well as the biotin surface concentration were varied systematically. The comparison of the AFM-data with the results of the fluorescence microscopy allows for important conclusion on the protein-protein binding, in particular concerning reproducibility, unspecific binding and protein resistance.

10:00am **BI-MoM6 A Comparison of Microcontact Printed and Solution Adsorbed Cytochrome c Protein Films on Indium Tin Oxide Electrodes**, ***A. Runge***, *S. Saavedra,* University of Arizona

The immobilization of proteins on a surface in a controlled way that retains their function is one of the challenges in making a functioning biosensor. Electrochemical biosensors use redox active proteins to impart selectivity to the electrode surface on which they are immobilized (either adsorbed or covalently attached). The orientation of proteins on the surface is presumed to be important for proper functioning of the device. We are investigating using microcontact printing as a way of immobilizing cytochrome c onto indium tin oxide electrodes in order to determine how the method of immobilization affects the orientation and function of the protein. We are presenting the results of three different methods for forming protein films on indium tin oxide that have been characterized and compared using cyclic voltammetry and X-ray photoelectron spectroscopy. Preliminary results from surface sensitive polarized spectroscopic studies will also be discussed. Protein films formed by adsorption of cytochrome c out of solution and by microcontact printing with both hydrophobic and hydrophilic PDMS are compared in terms of total and electrochemical surface coverage, standard reduction potential and rate of electron transfer with the ITO surface. Plasma treatment of the PDMS stamps, which makes them hydrophilic, dramatically increases the surface coverage of printed films to the level of solution adsorbed films. We have demonstrated that a redox active protein can be microcontact printed onto an electrode surface with its capability for direct electron transfer with the

surface intact. The total surface coverage of the different films were compared using X-ray photoelectron spectroscopy.

10:20am **BI-MoM7 Using Nanografting to Position with Predictable Orientation, De-novo Proteins on Gold**, ***Y Hu**, M. Case, G. McLendon, T.K. Vanderlick,* Princeton University*; D. Vanderah,* National Institute of Standards and Technology*; B. Nickel,* Princeton University*; M. Mrksich,* University of Chicago*; G.Y. Liu,* University of California, Davis*; G. Scoles,* Princeton University

An extensive research effort has been trying to make biosensors at the nanometer scale, especially selective detection devices with molecular recognition sites. We have approached this problem from a unique angle by using nanografting, which is to use an AFM tip to remove thiol molecules from a designated area of a self-assembled monolayer (SAM) while different thiol molecules from a contacting solution will self-assemble onto the exposed gold sites. By incorporating cysteine residues at the end of the peptide chains of [Fe(V@suba@L@subd@C-long)@sub3@]@super2+@, the proteins acquire the possibility to stand vertically on a gold surface. By using nanografting, the proteins could be patterned into islands about tens of nm wide and their properties could be measured in a differential way using the surrounding SAM as a reference. The height of these islands measures 3.2nm±0.4nm which, added to the 2.2 nm of the C@sub18@ SAM, corresponds well with the model height of the proteins. Effectively maximizing the signal-to-noise ratio of biosensors depends also on the ability to prevent protein nonspecific surface adsorption. It has been found that SAMs of thiols containing short oligomers of the ethylene glycol group prevent the adsorption of most proteins under a wide range of conditions. However the mechanism has not been clearly explained. It is observed that both SH(CH@sub2@)@sub11@(EO)@sub3@-OH and SH(EO)@sub6@-(CH@sub2@)@sub17@-CH@sub3@ reversibly compressed to half of their height under small imaging forces(0-10nN) in ethanol or 2-butanol. When the force is increased to over ~50nN, irreversible compression happened. Moreover, when the solution is changed to water mixture, the SH(EO)@sub6@-(CH@sub2@)@sub17@-CH@sub3@ is found to decrease its height significantly, and become much less compressible. By offering a model to explain the compressibility changes observed, we hope to offer some insight into the protein resistant properties of PEG-containing layers.

10:40am **BI-MoM8 Nanoscale Control of ECM Proteins for Cell Adhesion**, *H. Wang, L. Liu,* ***S. Chen**, T. Barker, H. Sage, B.D. Ratner, S. Jiang,* University of Washington

Osteopontin (OPN) is an important extracellular matrix protein shown to function in wound healing, inflammation and foreign body reaction and has been identified as a potential target for engineered biomaterials. The secreted protein acidic and rich in cystein (SPARC/osteonectin/BM-40) is associated with events characterized by changes in cell shape and mobility. In the work, we first report control of OPN orientation and conformation on charged self-assembled monolayers (SAMs) for cell adhesion. Our atomic force microscope (AFM) results show that the amount of adsorbed OPN on -COOH surface is slightly less than that on -NH2 surface. Results from in vitro cell adhesion assays show that on NH2 surface BAEC adhesion and spreading are more. By comparing these results, it is suggested that the orientation/conformation of OPN on -NH2 positively charged surface is more favorable for cell interactions than on -COOH negatively charged surface. Second, AFM is used to image the binding of OPN onto individual triple-helical collagen I monomer on freshly cleaved mica for the first time. We also use anti-OPN antibody to assist for better visualization. Analysis of AFM results clearly shows binding patterns of OPN to collagen I. Finally, the interactions of SPARC with ECM proteins, such as collagen I and fibronectin, are characterized and quantified using AFM and surface plasma resonance (SPR). Cell culture and adhesion assays are used to study SPARC as a modulator of the adhesive process of cells seeded on ECM proteins. The influence of SPARC-collagen I interaction is studied using smooth muscle cells while the influence of SPARC-fibronectin interaction is studied using endothelial cells.

11:00am **BI-MoM9 Activity of the Model Enzyme Urease Adsorbed on Different Colloidal Oxide Particles**, ***K. Rezwan**, J. Voros, M. Textor, L.J. Gauckler,* ETH Zurich, Switzerland

Whilst metal oxides are directly used for applications where their extreme hardness is necessary (e.g. femoral head replacement), most metallic biomaterials are themselves covered by a protective, stable oxide film such as titanium oxide on titanium. In these cases proteins only interact with the oxide film and not with the underlying metal. Closer investigations of the protein - oxide interface are therefore vital to the biomaterials field as it strives to make the transition from merely bio-inert to fully bioactive implant materials. It is assumed, that not only the amount of adsorbed protein but also its conformation is important for cell proliferation. A change of protein structure would hamper the cell receptors (situated within the cell membrane) to recognize the specific protein function. As a consequence, the cell would not adhere and proliferate on the preliminary adsorbed protein layer and not accept the adsorbed proteins as body own proteins but rather see them as intruders. This fatal mistake, made by the cell, is assumed to be one of the reasons which promotes local inflammation and tissue mutations. Assessing the conformational changes of a protein after adsorption is a delicate matter and can be measured by using for instance circular dichroism. In the case of enzymes, one can also measure the activity of the enzyme before and after adsorption. We used the model enzyme urease for our studies where the catalysis of urea was monitored by measuring the electrical conductivity as a function of time. The aim of this study was to compare the activity (and hence the conformational state) of urease after adsorption onto different colloidal oxide particles. We found that urease adsorbed onto TiO2 showed the highest activity and urease on Al2O3 the lowest. The measurements showed also an adsorption time dependency, which indicated further conformational changes after adsorption.

11:20am **BI-MoM10 In situ Real-time Atomic Force Microscopy Studies of Lysozyme and RR02 Protein Crystal Growth at Surfaces**, *T.R. Keel, S. Allen, M.C. Davies, C.J. Roberts,* ***S.J.B. Tendler**, P.M. Williams,* University of Nottingham, UK

The successful application of crystallography to fields such as structural biology and rational drug design has been largely due to the availability of single crystals of the macromolecule of interest. However, relatively little is understood about the fundamentals of macromolecular crystal growth. Here, we have utilized the technique of atomic force microscopy (AFM) to study protein growth at surfaces. Two different protein systems, at both the micro- and nanometre scale have been investigated. The first is a model system, lysozyme. We have investigated two polymorphs of the lysozyme crystal and present data concerning the effect of supersaturation (@sigma@) on the growth rates and mechanisms of growth of the crystals. Molecular resolution studies have also been carried out and the observed periodicities are in good agreement with the known unit cell dimensions. Also presented are preliminary results from a second protein system, RR02, which is a response regulator protein found in streptococcus pneumoniae. RR02 is relatively straightforward to crystallize, but unlike lysozyme, many of the grown crystals diffract poorly and x-ray studies yield little structural data. We have used the AFM to investigate these crystals and compare the results with the lysozyme studies.

Biomaterial Interfaces
Room 307 - Session BI-MoA

Non-fouling Surfaces and Biolubrication
Moderator: D.G. Castner, University of Washington

2:00pm **BI-MoA1 Towards the Prevention of Protein Adsorption, and Bacterial and Cell Adhesion by Optimised Surface Modification**, ***P. Kingshott***, *J. Wei,* Risoe National Lab, Denmark*; H. Thissen,* CSIRO Molecular Science, Australia*; N. Gadegaard,* Univ. of Glasgow, UK*; D. Selmeczi,* Risoe National Lab, Denmark*; L. Gram, D. Bagge-Ravn,* Technical Univ. of Denmark, Denmark*; N.B. Larsen,* Risoe National Lab, Denmark*; H.J. Griesser,* University of South Australia, Australia **INVITED**

A non-fouling surface is still elusive since problems such as bacterial colonisation on medical devices and food processing equipment, and failure of implants caused by clotting and foreign body reactions are still existent. Surface modification with PEG or PEO is the most effective way of reducing protein adsorption, and bacterial and cell adhesion (also called bio-adhesion). The best reports show that protein adsorption can be reduced to a fraction of the uncoated surface or even prevented. However, so far reductions in bacterial adhesion by PEG surfaces have only been marginally successful (up to 1 or 2 orders of magnitude). Why is this the case? Can surfaces that prevent protein adsorption also prevent bacterial and cell attachment? Can theoretical predictions of a non-fouling surface ever be put into practice by design of the perfect surface? In order to answer these questions it is necessary to be able to generate stable PEG layers with sufficiently high graft density and uniformity to provide the optimal steric repulsive barrier against bio-adhesion. In addition, to make the claim that a surface is non-fouling depends on one being able to detect protein adsorption below the threshold where no subsequent events can occur (such as bacterial adhesion). In this presentation some of these issues will be discussed. Surface modification based on plasma polymerisation and wet chemical methods are used to provide reactive groups for PEG grafting, confirmed by surface derivatisation reactions and surface analysis including XPS and ToF-SIMS. PEGs are grafted at their lower critical solution temperature (LCST) for maximal surface coverage. In addition, highly sensitive and specific surface mass spectrometry analysis (i.e. ToF-SIMS and surface-MALDI) is shown to be both very useful at detecting ultra-low levels of protein on the best PEG surfaces. Furthermore, the ability of PEG surfaces to prevent protein adsorption is heavily dependent on the size and type of protein.

2:40pm **BI-MoA3 Factors that Determine the Protein Resistance of Oligoether Self-assembled Monolayers - Internal Hydrophilicity, Terminal Hydrophilicity and Lateral Packing Density**, *S. Herrwerth, W. Eck,* ***M. Grunze***, University of Heidelberg, Germany

Protein resistance of oligoether self-assembled monolayers (SAMs) on gold and silver surfaces has been investigated systematically in order to elucidate structural factors that determine whether a SAM will be able to resist protein adsorption. Oligo(ethylene glycol) (OEG), oligo(propylene glycol) and oligo(trimethylene glycol) terminated alkanethiols with different chain length and alkyl termination were synthesized as monolayer constituents. The packing density and chemical composition of the SAMs were examined by XPS spectroscopy; the terminal hydrophilicity was characterized by contact angle measurements. IRRAS spectroscopy gave information about the chain conformation of specific monolayers; the amount of adsorbed protein compared to alkanethiol monolayers was determined by ellipsometry. We found several factors that can suppress the protein resistance of oligoether monolayers. Monolayers with a hydrophobic interior such as those containing oligo(propylene glycol) show no protein resistance. The lateral compression of oligo(ethylene glycol) monolayers on silver generates more highly ordered monolayers and may cause decreased protein resistance, but does not necessarily lead to an all-trans chain conformation of the OEG moieties. Water contact angles higher than 70Â° on gold or 65Â° on silver reduce full protein resistance. We conclude that both internal and terminal hydrophilicity favor the protein resistance of an oligoether monolayer. It is suggested that the penetration of water molecules in the interior of the SAM is a necessary prerequisite for protein resistance. We discuss and summarize the various factors and the balance of forces which are critical for the functionality of "inert" organic films.

3:00pm **BI-MoA4 Use of QCM-D to Analyze Thin Polymer Films at Interfaces**, ***E.F. Irwin***, *J Ho, K.E. Healy,* University of California, Berkeley

An interpenetrating polymer network (IPN) of acrylamide (AAm) and poly(ethylene glycol) (pEG) was designed that can be covalently bound directly onto metal oxide and polymer surfaces via photoinitiated free radical polymerization. A p(AAm-co-EG) with acrylic acid (AA) was also designed to allow further functionalization of the IPN surface with a diamino pEG spacer arm (pEG-NH@sub 2@).@footnote 1@ In this study, we are employing a quartz crystal microbalance with dissipation (QCM-D) (qsense) to monitor the IPN swelling and protein adsorption behavior in phosphate buffered saline (PBS), pH 7.4. QCM-D crystals coated with SiO@sub 2@ and TiO@sub 2@/Ti were modified with IPNs of p(AAm-co-EG), p(AAm-co-EG/AA), and p(AAm-co-EG/AA) + pEG-NH@sub 2@. The Sauerbrey relationship was used to calculate a thickness of 48nm for a dry film of p(AAm-co-EG/AA). QCM-D thickness data can be compared to a dry IPN thickness of 17nm determined previously by spectroscopic ellipsometry.@footnote 1@ A Kelvin-Voight model of viscoelasticity was used to interpret frequency and dissipation data of the hydrated films over the swelling period. Modeling the swelling data of a p(AAm-co-EG/AAc) IPN gave an initial hydrated thickness of 101nm (after 2 minutes) and a final swollen thickness of 150nm. The shear modulus of the film ranged from 285 to 365kPa and the viscosity ranged from 6.7E-3 to 8.6E-3kg/ms according to the model. One limitation of this model is that one single density of the IPN surface is assumed (in this case a density of 1.1g/cm@super 3@) over the entire swelling period. These IPN surfaces minimize the adsorption of the protein fibrinogen that has a role in thrombosis. The QCM-D provides unique and complementary information to other surface analytical techniques (i.e. AFM, XPS) for understanding the behavior of thin polymer films at interfaces. @FootnoteText@ @footnote 1@ Bearinger, JP, et al., J. Biomat. Sci. Polym. Ed., 9 (7) 1998.

3:20pm **BI-MoA5 Non-Fouling Surfaces: Their Use and Study by Matrix-Assisted Laser Desorption / Ionization Mass Spectrometry**, *G.R. Kinsel,* ***J. Zhang***, *R.B. Timmons,* The University of Texas at Arlington

Matrix-Assisted Laser Desorption / Ionization mass spectrometry (MALDI MS) has emerged in recent years as a powerful method for the mass spectrometric analysis of a wide range of biomolecules including proteins, oligonucleotides, polysaccharides, etc. Advantages of this analytical approach include simplicity of sample preparation, high analysis speed and high sensitivity. Recently MALDI-MS has been used in the characterization of non-fouling surfaces and related mechanistic studies in our group. Specifically, non-fouling coatings are applied to MALDI sample targets using a variety of published approaches including PEO chemical modification of polyurethane and pulsed plasma deposition of tetraethylene glycol dimethylether. From a practical standpoint these surfaces are shown to significantly lower the limit of detection (to sub-femtomolar quantities) in a MALDI experiment, presumably by reducing the amount of protein lost to surface-binding interactions. This influence is revealed by the acquisition of MALDI standard curves for a variety of peptides and proteins using methods previously established in our group. Additional studies of the influence of various MALDI parameters, including matrix solvent, pH, and ionic strength and various surface properties, primarily contact angle, have been performed to reveal relationships between, for example, surface hydrophilicity and protein binding, peptide/protein size and protein binding and elution solvent properties and protein binding. These studies offer useful experimental insights into various proposed mechanisms of non-fouling behavior.

3:40pm **BI-MoA6 Comparison of Immunoassay Blocking Strategies on Metal Oxide Substrates**, ***A.N. Scribner***, *C.L. Cole, R.J. Colton, L.J. Whitman,* Naval Research Laboratory

We have developed an alumina filter-based immunosensor that is 10 times faster and ~3 orders of magnitude more sensitive than an analogous microtiter well-based format. The assay is based on a standard sandwich immunoassay but uses magnetic microbeads and magnetic forces to differentiate between specific and nonspecific interactions. The combined use of magnetic force discrimination with PEG-based surface chemistries that minimize nonspecific binding forces result in a demonstrated specificity of >98%. Additionally, a more traditional blocking agent can also be added to compensate for lot-to-lot variability in the surface chemistry of commercially available alumina membranes. However, immunoassays on metal oxide supports not based on electrochemical detection are uncommon, so comparatively little is known about the effectiveness of different blocking agents for such surfaces. We examine agents typically used to block polystyrene plates for their relative effectiveness at blocking

PEGylated and non-PEGylated alumina membranes, including detergents, proteins, hydrophobic, and hydrophilic molecules. The effectiveness of each substance as a blocker is determined quantitatively by measuring the amount of IgG-HRP remaining after incubation on a pre-blocked surface. Our results suggest that traditional reagents such as gelatin or BSA do not have the same ability to block nonspecific binding on PEGylated alumina as on polystyrene, and that casein and charged reagents such as SDS may be more appropriate choices for the blocking of modified metal oxide surfaces. Supported by ONR and the DoD JSTPCBD. ANS and CLC are employees of Nova Research, Inc., Alexandria, VA.

4:00pm **BI-MoA7 Lubricating with Water: Biomimetic Additives**, *M. Müller, S. Lee,* ETH-Zürich, Switzerland; *X. Yan, S.S. Perry,* University of Houston; ***N.D. Spencer,*** ETH-Zürich, Switzerland **INVITED**

Nature often relies on surface-bound, brush-like structures to impart lubricity to natural surfaces (joints, G.I. tract, lungs) in an aqueous environment. These generally consist of polysaccharides, which are frequently charged and coordinate a large amount of water. We have found that another heavily hydrated brush-forming system: poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG), can impart lubricity to inorganic surfaces, such as silicon, glass and steel, in an aqueous environment. A clear dependence on polymer architecture can be observed, which is manifested on both nano- and macro-scales, as determined by AFM and tribometer measurements, respectively.

4:40pm **BI-MoA9 Boundary Lubrication Properties of Bio- and Synthetic Polymers Containing Poly- and Oligosaccharides**, ***S. Lee***, *G. Kilcher, N.D. Spencer,* ETH-Zürich, Switzerland

In this study, we have investigated the boundary-lubrication properties of aqueous solutions of natural and synthetic polymers possessing poly- and/or oligosaccarides as an additive to reduce interfacial friction forces. As natural polymers containing poly- and oligosaccarides, we have chosen porcine gastric mucin (PGM) as a standard material responsible for bio-lubrication. Mucins are large and complex glycoproteins composed of a linear polypeptide and polysaccarides side-chains. Due to their aggregation or polymerization, often involving gel formation, mucins are known to form a protective layer between the lumen and the cell surface. Mucins possess a structure involving a combination of hydrophilic and hydrophobic domains with high molecular weight. The boundary lubrication properties of PGM-containing aqueous solutions have been investigated on hydrophobic tribo-pairs, such as self-mated poly(dimethylsiloxane) (PDMS). To more systematically investigate the role of poly- and/or oligosaccarides for water-based lubrication, we have synthesized block copolymers consisting of a polypeptide backbone, e.g. poly(L-lysine), and oligosaccarides with well-defined structure and chemistry. For this model system, we have selected various oxide surfaces as a tribo-pair. Both macroscopic- and molecular-scale sliding contact have been investigated employing pin-on-disk tribometry and atomic force microscopy respectively. The frictional properties of the selected tribosystem have been measured as a function of pH and ionic strength/type of the aqueous lubricant solution. The observed changes of the lubrication properties of both bio- and synthetic polymers as a function of pH and ionic strength are discussed in terms of the corresponding changes of conformation and adsorption behavior.

5:00pm **BI-MoA10 The Role of Polysaccharides in Bacterial Adsorption: A Chemical Perspective**, ***K.T. Queeney***, *J.W. Clemens, C. Royce,* Smith College

While it is well known that extracellular polysaccharides influence the adhesion properties of a range of encapsulated bacteria, studies of the adsorption properties of these polysaccharides have been largely limited to investigations of their conformational and/or mechanical properties.@footnote 1@ Xanthan, a model bacterial polysaccharide, has been well studied in the solution phase and therefore provides a useful starting point for understanding, at a molecular level, what influences the adsorption properties of these large and complex molecules. We have used surface infrared spectroscopy to investigate the adsorption of xanthan on a variety of surfaces that exhibit both varying hydrophobicity and a range of chemical terminations. While a previous study of polysaccharide adsorption on oxide surfaces focused only on hydrogen-bonding behavior as evidenced by the OH-stretching region,@footnote 2@ we find that the carbonyl stretching region shows marked changes in the local chemical environment of these moieties, suggesting that they interact strongly with the surface. Furthermore, xanthan's similar affinity for hydrophobic and hydrophilic surfaces provides evidence that polysaccharide/surface interactions must include non-hydrogen bonding effects. @FootnoteText@ @footnote 1@See for example T. A. Camesano and K. J. Wilkinson, Biomacromolecules 2, 2001, 1184-1191.@footnote 2@B. A. Jucker, H. Harms, S. J. Hug and A. J. B. Zehnder, Coll. Surf. B 9, 1997, 331-343.

Applied Surface Science
Room 324/325 - Session AS-TuM

Image Analysis and Polymer Characterization
Moderator: F.A. Stevie, North Carolina State University

8:40am **AS-TuM2 Advances in Chemical Imaging: NanoSAM and NanoESCA**, ***J. Westermann***, *G. Schaefer, D. Funnemann, M. Maier,* Omicron NanoTechnology, GmbH, Germany

Electron spectroscopy has been a proven tool for scientific applications for decades. Challenging new applications are emerging from the fields of semiconductor and nanotechnology research and a key issue for these areas is the non-destructive imaging of sensitive structures with nanoscale dimensions. Characterization of their chemical composition and electrical properties goes hand in hand with this. We report on the development of two novel electron microscopes for chemical imaging that meet these requirements, especially for lateral image resolution in the nanoscale range. I. NanoSAM We present electron optical concepts and first results of a truly UHV compatible SEM column designed to meet the requirements for high-resolution with high beam currents. Performance checks on nanostructured samples demonstrate spot sizes below 3 nm at 15 keV beam energy and better than 5 nm at 3 keV, with sample currents being suitable for Auger electron analysis. Latest static Auger and SAM results, demonstrating the outstanding spatial resolution, will be shown. II. NanoESCA We present a parallel imaging electron microscope with an integrated high-resolution energy filter for Imaging XPS. It consists of a Photo Emission Electron Microscope (PEEM) with a large angular acceptance and an aberration corrected energy filter. The microscope allows for imaging with chemical contrast (Imaging ESCA) by energy filtering of photoelectron images. The analyzed energy of the photoelectron images ranges from threshold photoemission to 1600 eV. The instrument has been characterized with laboratory and synchrotron excitation sources. The spatial resolution limit measured so far in imaging ESCA mode is about 150 nm. The measured energy resolution follows the theoretical calculations. We show XPS spectra and energy filtered image series (video sequences) of: AlGaAs heterostructures, microstructured Au/Si and Ag/Ta samples.

9:00am **AS-TuM3 Correlation of XPS and AFM Images for Polymer Blends**, ***J. Farrar***, *K. Artyushkova, J.E. Fulghum,* University of New Mexico*; F. Xu, N. Bantan, J. Khan,* Kent State University

Multi-technique analysis of heterogeneous polymer samples, based on AFM and XPS imaging, will provide a more complete picture of the sample under study than either technique alone. AFM provides both topographical and phase contrast information on the nanometer scale, but no chemical information is provided. Imaging XPS provides elemental and chemical information on the micron scale. A method to chemically identify the observed phases in AFM through correlation with quantitative XPS imaging will be discussed. Correlating the data from both techniques involves resizing, image alignment, resolution matching, and classification methods. The approach will be applied to a patterned polymer surface of known properties for validation and then to heterogeneous polymer blends of polystyrene/polybutadiene for phase identification. This project represents one aspect of the Active Knowledge Mesh Model (AKM) that is currently under development in our laboratories. AKM is a comprehensive image analysis system that integrates data from different techniques into a realistic three-dimensional model, visualizing structure and morphology, in multicomponent heterogeneous samples. This work has been partially supported by NSF ALCOM (DMR89-20147), NSF CHE-0113724, NSF IGERT CORE and UNM.

9:20am **AS-TuM4 XPS and Confocal Microscopy Data Fusion for Polymer Characterization**, ***K. Artyushkova***, *J.E. Fulghum, J. Fenton, J. Farrar,* The University of New Mexico*; J. Khan, F. Xu, N. Bantan,* Kent State University

Advances in materials development require a three-dimensional characterization of complex polymeric samples in terms of both chemical structure and morphology over feature sizes ranging from nanometers to millimeters. Correlating information from multiple techniques is one method for the development of a comprehensive 'picture' of the material under study. New opportunities for multi-technique correlations arise from the improved spatial resolution and decreased acquisition times now available in a variety of imaging methods. Imaging XPS and confocal microscopy (CM) are complementary techniques that, in combination, allow for the visualization of the internal structure of heterogeneous polymer samples. Quantitative chemical information that is readily available from the surface sensitive XPS images and spectra will be used to extract quantitative data from confocal images, which are acquired from different depth levels throughout the sample. The XPS and CM data sets can then be 'fused' to provide a surface-to-bulk visualization of changes in polymer chemistry. This work has been partially supported by NSF CHE-0113724 and NSF IGERT CORE program.

9:40am **AS-TuM5 Solvent Enhanced Surface Modification of Polymers Accompanying AFM Tip Induced Mechanical Stresses**, ***F. Stevens***, *R. Leach, J.T. Dickinson,* Washington State University

The response of thin polymer films and bulk surfaces to combined stress and solvent is important for applications such as protective barriers (e.g., various wrappings), in controlled drug release from polymer hemispheres, resists for lithography, and nanometer scale surface modification. Over a wide range of normal forces, when a polymer is scanned by an AFM tip in contact mode in a solvent, material is not worn away, but rather the polymer surface expands forming a nanometer scale "protrusion" at and surrounding the scanned location. Furthermore, for sub-micron scan areas one observes a series of parallel ridges (moguls) perpendicular to the fast scan direction. Previous reports of the formation of raised material have nearly all been in air, required long times or very high forces to form; little evidence has been presented for the mechanism of formation. We have made a detailed study of protrusion formation and raised ridges using poly(methyl methacrylate) in alcohol based solvents. In addition to scanning in air, we have scanned the polymer surface of several molecular weights in four solvents with dramatic differences in response. We present evidence that both plastic deformation and tip induced swelling play major roles in the observed polymer surface modification by AFM. Extending this work, we have added temperature as a parameter, performing the tip stimulation at temperatures from 0 - 120 C. Major changes in the structures formed occur at higher temperatures due to increased solvent mobility and polymer mechanical properties with temperature.

10:00am **AS-TuM6 SF5+ Ion Beam Damage of Poly(Acrylates) Studied using Time of Flight Secondary Ion Mass Spectrometry**, ***M.S. Wagner***, *G. Gillen,* National Institute of Standards and Technology

Recent advancements in instrumentation for Secondary Ion Mass Spectrometry (SIMS) have focused on the development of polyatomic primary ion sources. Polyatomic ions have been shown to increase the secondary ion yields of molecular ions from organic materials when compared with monoatomic ions of similar mass. Furthermore, some polymer films, notably poly(methyl methacrylate) and poly(ethylene glycol), display the unusual characteristic of maintaining their characteristic molecular ion signals after extended polyatomic ion bombardment. This study focuses on the effect of the chemical structure of the polymer on its stability under extended SF5+ ion bombardment. The damage of spin cast polymer films by 5 keV SF5+ was studied using positive and negative ion static Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS). A class of poly(acrylate) polymers with systematic changes to the monomer structure were investigated to determine the effects of polymer structure on the stability of their characteristic ion signals under 5 kV SF5+ bombardment in the ion dose range from 2.5 x 10@super 13@ - 5 x 10@super 14@ SF5+ ions/cm@super 2@. Preliminary results have shown that poly(methacrylic acid) and poly(methyl methacrylate) were stable under extended SF5+ bombardment while poly(methyl acrylate) was not, suggesting a role for the methyl group on the central carbon in the stabilization of the polymers under SF5+ bombardment. Monte Carlo calculations using the SRIM software@footnote 1@ show that the penetration depths of fluorine and sulfur ions in these polymers were the same despite the different damage characteristics, highlighting the importance of the chemical structure of the polymer on its stability under polyatomic ion bombardment. This study describes the breadth of applicability of SF5+ sputtering to the depth profiling of polymer films. @FootnoteText@ @footnote 1@ More information on this program can be found at http://www.srim.org.

10:20am **AS-TuM7 Time-of-Flight Secondary Ion Mass Spectrometry of Ordered Polymeric Monolayers: Effect on Tertiary Structures**, *J.A. Gardella, Jr.,* ***R. Rey-Santos***, State University of New York at Buffalo

The study of polymer surface structures has become an important topic in surface chemistry. Poly(dimethylsiloxane) (PDMS) plays very important roles in biological and pharmaceutical applications. A similar study of poly(methylmethacrylate) published by Nowak et al in Analytical Chemistry in 2000 was used as a model. Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) is one of the most powerful techniques for

polymer study. TOF-SIMS was used to study the fragmentation mechanism of this polymer. Using a statistical chain breaking model of PDMS helped us to understand the fragmentation pattern obtained in the spectra. The Langmuir-Blodgett (LB) technique helps us to prepare a molecular monolayer at the air/water interface. The LB technique was used to prepare well-ordered monolayers. Reflection Absorption Fourier Transform Infrared Spectrophotometry (RA-FTIR) is an ideal technique for surface study to obtain information about molecular structures. It has been used to compare results from amorphous, solution cast films to that from ordered, LB films of PDMS. An ion formation mechanism for PDMS was investigated to get information of the long-range forces between polymer chains.

10:40am **AS-TuM8 Grazing Incident X-ray Diffraction Study of Poly(alkylthiophene) Thin Films on Various Substrates**, *M. Morita, T. Koga, H. Yakabe, S. Sasaki,* Kyushu University, Japan*; O. Sakata,* JASRI, Japan*;* ***A. Takahara,*** Kyushu University, Japan

The crystalline aggregation states of organic semiconductor molecules in thin films might play an important role in the performance of organic semiconductors. The purpose of this study is to reveal the crystalline orientation and crystallinity of spin-coated poly(3-hexylthiophene)[P3HT] on Si-wafer substrate at the nearã€€surface region and internal phase. The surface crystalline states of P3HT thin film were studied with synchrotron radiation in-plane grazing incidence x-ray diffraction GIXD. Surface modification of Si-wafer with perfluorohexylethyltrimethoxysilane prior to P3HT coating resulted in the preferential orientation with the alkyl side chains normal to the substrate surface. In contrast, the surface modification of Si-wafer with vacuum ultraviolet(VUV)-ray with wave length of 172nm or n-octadecyltrimethoxysilane resulted in the orientation with the alkyl side chains parallel to the substrate.

11:00am **AS-TuM9 Study of Water-Induced Reorganization of Amphiphilic Graft Copolymers Containing Poly(2-hydroxyethyl methacrylate) and Polydimethylsiloxane by Angle-dependent X-ray Photoelectron Spectroscopy**, ***L. Chen,*** State University of New York at Buffalo*; A.P. van Bavel,* Eindhoven University of Technology, The Netherlands*; J.A. Gardella,* State University of New York at Buffalo

The free air surface composition and water-induced reorganization of a series of amphiphilic graft copolymers consisting of hydrophilic poly(2-hydroxyethyl methacrylate) [poly(HEMA)] backbones and hydrophobic poly(dimethylsiloxane) (PDMS) side chains prepared by both photoinduced-radical and anionic polymerization techniques were studied by angle-dependent X-ray Photoelectron Spectroscopy (XPS). Copolymers with broad molecular weight distributions (MWD; e. g. Mw/Mn = 3.0) were prepared by the radical copolymerization technique. Copolymers with narrow MWD (e. g. Mw/Mn = 1.1) were prepared by the anionic copolymerization technique. The free air surface composition of copolymers was studied as a function of MWD, PDMS bulk content and PDMS graft length. It was found that anionically prepared copolymers with narrow MWD have higher PDMS surface concentration than radically prepared copolymers with broad MWD. PDMS surface concentration increased with increasing PDMS bulk content. For a given PDMS bulk content, a longer PDMS graft chain gave a surface richer in siloxane. The water-induced reorganization of copolymers was studied as a function of MWD, PDMS graft length and water exposure time. The dry films were exposed to water (hydration) and then frozen in a nitrogen gas purge to preserve the surface composition during XPS analysis. The XPS results showed both MWD and PDMS graft length influenced the extent of the graft chain reorganization at the surface.

11:20am **AS-TuM10 The Role of Polymer Architecture and Environmental Humidity on the Interfacial Conformation and Properties of Surface-adsorbed Poly(l-lysine)-graft-poly(ethylene Glycol) Co-polymer**, ***F. Assi,*** *S. Pasche, L. Feuz, N.D. Spencer, M. Textor,* ETH Zurich, Switzerland

The development of protein-resistant surfaces is of central interest in the context of biosensor chip development and for the design of medical implants in contact with blood. Self-organized films of PEG based co-polymers on different oxide surfaces are one system that has been investigated by a variety of surface characterization techniques in order to establish systematic correlations between the polymer composition/structure, interface architecture and interaction with protein-based biological media. A class of co-polymeric molecules of special interest is based on a poly(L-lysine) backbone, charged positively due to the presence of protonated amine groups at a neutral pH, and grafted with poly(ethylene glycol) side chains (short: PLL-g-PEG). Although the protein-resistant properties of these films have already been demonstrated, little is known about the effect of the co-polymer interfacial architecture on the resulting protein resistance. One of the most important factors turns out to be the polymer conformation in the adsorbed state, which depends not only on the polymer architecture but also on the environment the polymer, is exposed to. We report results that elucidate the surface conformation of PLL-g-PEG of different molecular architecture at various humidity levels in air as well as in contact with aqueous solutions, studied by means of atomic force microscopy (AFM, for the polymer conformation and adhesion properties) and ellipsometry (ELM, for the layer thickness). AFM force-distance measurements in compression between a 5-µm SiO@sub 2@ sphere and a PLL-g-PEG-coated substrate showed a good correlation with the architecture of the polymer. Furthermore, tensile-mode (pull-off) AFM studies were used to quantify the adhesion strength level of the polymeric molecules at oxide surfaces. The experimental results for different polymers were finally compared to the results of self-consistent field calculations.

11:40am **AS-TuM11 Ultra-fast Laser Ablation as a Facilitator for Depth-dependant Characterization of High Pigment Volume Concentration Organic Coatings**, ***L.T. Keene,*** *C.R. Clayton, G.P. Halada, T. Fiero,* State University of New York at Stony Brook

The strong dielectric nature of most organic coatings, particularly those used on an industrial scale, presents the scientific investigator considering a depth-profiling approach to chemical characterization of such organic coatings with a serious challenge. The ultra-fast optical phenomenon of femtosecond laser ablation presents one possible solution to such a problem. An apparent athermal, non-selective ablation process becomes possible when the pulse temporal scale reaches the femtosecond (10@super -15@ s) level. This remarkable property of femtosecond-class lasers enables the multi-layer. removal of organic/inorganic composite coatings for the purpose of chemical characterization as a function of coating depth@footnote 3,4@ If proven experimentally, this capability becomes attractive when considering the depth-analysis of materials that demonstrate either strong dielectric properties (and, hence, resist depth profiling via traditional charged particle beam methods) or are chemically inhomogeneous in nature (which generally cause problems such as selective sputtering). This talk will focus on the experimental use of ultra-fast laser ablation for the facilitation of depth-profiling high solids organic coatings with particular emphasis placed on military application two-component solvent-based polyurethane coatings containing a variety of inorganic pigments and fillers.@footnote 1,2@ The high dielectric and chemically inhomogeneous natures of these coatings pose a demanding application for uniform laser removal without chemically modifying the host material. Cross-sectional analysis of the materials via Scanning Electron Microscopy (SEM) / Energy Dispersive Analysis of X-rays (EDS) will be shown. Ablation of the aforementioned materials was conducted both in atmosphere as well as high vacuum. Chemical modification of host material due to the ablation process will be discussed via the results of Fourier Transform Infrared Spectroscopic analysis of ablated material before, and after, ablation. Morphological features of ablated regions collected via high-resolution scanning confocal profilometry as a function of the processing parameters, and how these features limit the removal depth resolution, will be shown. @FootnoteText@ @footnote 1@ S. McKnight, J. Beatty, Mechanisms of Military Coatings Degradation, ARL Weapons & Materials Directorate, (1999)@footnote 2@ 1. C.R. Hegedus, et. al., "A Review of Organic Coating Technology for U.S. Naval Aircraft," Journal of Coatings Technology, Vol. 61, No. 778, pp 31-42, (1989)@footnote 3@ L. Keene, G. Halada, C. Clayton, S. McKnight, W. Kosik, "Novel Techniques for the Investigation of Long-term Photo-degradation of Mult-layer Polymer Coatings," in State-Of-The-Art Application of Surface and Interface Analysis Methods, The Electrochemical Society Proceeding Series, Pennington, NJ, (199th Electrochemical Society Meeting; Washington D.C.) (2001)@footnote 4@ A.A. Serafetinides, M.I. Makropoulou, C.D. Skordoulis, A.K. Kar Appl. Surf. Sci. 42-56 (2001) 180

Biomaterial Interfaces
Room 307 - Session BI-TuM
Cell/Surface Interactions

Moderator: K.E. Healy, University of California, Berkeley

8:20am **BI-TuM1 Modulation of Vascular Smooth Muscle Cell Behavior by Tuning Substrate Compliance**, *X.Q. Brown,* ***J.Y. Wong,*** Boston University

During the development of vascular occlusive disease, abnormal vascular smooth muscle cell (VSMC) proliferation and deposition of extracellular matrix leads to hardening of the vessel. Recent studies have shown that

changes in substrate compliance affect cell adhesion, migration and differentiation in several different cell types. However, effects of substrate stiffness on the behavior of VSMCs have not yet been investigated. Using polydimethyl siloxane (PDMS), we are able to create substrates with Young's modulus ranging from 3x10@super 4@ Pascal (Pa) to 2x10@super 6@ Pa, which is the range of elasticity that has been reported for a human aorta. We found that changes in substrate compliance affect the behavior of VSMCs. There is a 0.7 fold increase in cell number attached to stiff substrata (2x10@super 6@ Pa) compared to soft substrata (3x10@super 4@ Pa) and a 0.8 fold increase in cell area on stiff substrata. However, the rate of VSMC proliferation on stiff substrata is only 66% that of VSMC on soft substrata. Our results show that VSMCs are capable of sensing and responding to changes in substrate compliance in a range that is physiologically relevant, and PDMS is a useful tool to study the effect of substrate compliance on cell behavior. Our results support our hypothesis that during the development of vascular occlusive disease, changes in VSMC behavior leads to changes in the biochemical and biophysical properties of the vessel, which in turn influence the behavior of VSMCs.

8:40am **BI-TuM2 Mechanical and Biochemical Analyses of Cell Adhesion Strengthening Using Micropatterned Substrates**, ***N.D. Gallant***, *A.J. García,* Georgia Institute of Technology

Cell adhesion to fibronectin (FN) involves integrin binding and subsequent adhesion strengthening, which includes integrin clustering, interactions with cytoskeletal and signaling components to form focal adhesions (FA), and cell spreading. We applied micropatterning methods to control FA size and position to analyze the contributions of FA assembly to adhesion strength. Microcontact printing was used to pattern alkanethiol self-assembled monolayers into arrays of circular adhesive islands (2, 5, 10, 20 μm dia) with a non-adhesive background.@footnote1@ NIH3T3 fibroblasts adhered to FN-coated islands and remained constrained to the patterns presenting a nearly spherical morphology. Cells assembled robust adhesive structures that localized to the micropatterned islands and contained typical components of FA. Cell adhesion strength to FN-coated micropatterned islands was quantified using a spinning disk device that applies a well-defined range of hydrodynamic forces to adherent cells.@footnote2@ Adhesion strength exhibited significant time- and adhesive area-dependent increases. Comparison of experiments for equivalent contact areas showed a 9-fold increase in adhesion strength over time, independent of cell spreading. Bound integrins were quantified using a cross-linking/extraction/reversal biochemical technique.@footnote3@ Significant area dependence was also seen in integrin binding on micropatterned substrates and a correlation between increasing integrin binding and adhesion strength was observed. These results demonstrate that FA assembly, independently of changes in cell morphology, contributes significantly to adhesion strengthening. This work provides an experimental framework for the functional analysis of FA components in adhesive interactions. @FootnoteText@ @footnote 1@N.D. Gallant et al., Langmuir 18, 5579-5584, 2002.@footnote 2@A.J. García et al., J. Biol. Chem. 273, 10988-10993, 1998.@footnote 3@A.J. García et al., Mol. Biol. Cell 10, 785-798, 1999.

9:00am **BI-TuM3 The Use of XPS, SIMS, and Immunostaining to Examine the Behavior of Extracellular Matrix upon Cell Detachment from a Smart Polymer**, ***H.E. Canavan***, *X. Cheng, B.D. Ratner, D.G. Castner,* University of Washington

The temperature-dependent behavior of poly(n-isopropylacrylamide) (NIPAM) is directly transmitted to cells cultured on these surfaces. At culture temperatures, cells behave similarly to those on tissue culture polystyrene (TCPS); after being cooled to room temperature, cells cultured on NIPAM spontaneously detach as contiguous sheets. In comparison, cells grown atop the TCPS surface remain attached for hours or days, requiring the use of enzymatic digestion or physical scraping to detach them. In addition, cell sheets detached from NIPAM surfaces appear to retain their function upon transfer to another growth surface, possibly due to the concurrent detachment of at least one protein of the Extracellular Matrix (ECM), fibronectin (FN). However, the extent to which ECM detaches from the NIPAM surface has remained unknown. We present a thorough examination of the cellular response to NIPAM using X-ray Photoelectron Spectroscopy (XPS), Secondary Ion Mass Spectrometry (SIMS), and immunostaining. XPS is used to make a quantitative comparison of the amount of protein atop NIPAM after cell removal. The primary proteins of the ECM (FN, laminin, and collagen) are examined using immunostaining to determine which of the ECM proteins studied lift off with the cellular layer. In addition, SIMS is used to identify the presence and identity of proteins left at the NIPAM surface after liftoff. Finally, the low-temperature liftoff technique is compared to other traditional cell removal protocols. Our SIMS, XPS, and immunoassay results suggest that low-temperature liftoff of the cell monolayer from the NIPAM surface is accompanied by the majority of the components of the ECM.

9:20am **BI-TuM4 Neurite Outgrowth on Well Characterized Surfaces: Chemically and Spatially Controlled Fibronectin and RGD Substrates**, ***Z Zhang***, *R. Yoo, M. Wells, T.P. Beebe, Jr.,* University of Delaware*; R. Biran, P. Tresco,* University of Utah*; J. Hyun, W. Jun, A. Chilkoti,* Duke University

Study of axonal growth and ligand-receptor interactions requires specificity and careful characterization of the biomaterial substrates to which the neurons bind. Without highly specific surface characterization, it would be impossible to predict the effects of ligand surface density, spatial distribution, and conformation on the outgrowth of a neuron. Here we report two different methods of surface modification (a heterobifunctional crosslinker and Pluronics @super TM@) for immobilization of fibronectin (FN) and fibronectin-derived RGD-containing peptides to the substrates. Proteins and peptides were immobilized to glass surfaces at different concentrations. Various surface analytical techniques, such as contact angle, x-ray photoelectron spectroscopy (XPS), and time-of-flight secondary ion mass spectrometry (TOF-SIMS) were used for analysis of the substrates at each step of the two different chemistries involved. After immobilization of fibronectin and RGD-containing oligopeptides, the modified surfaces were plated with dorsal root ganglia neurons of the rat. Neuron outgrowth rates on the various surfaces were measured and different bioactivity was observed on different modified surfaces. In order to spatially control neurite outgrowth on the substrates, patterned and gradiently FN-covered surfaces were synthesized and tested for bioactivity. An amphiphilic comb polymer presenting oligoethylene glycol side-chains was used to create microcontact-printed patterned surfaces because of its excellent protein repellant and cell resistance properties.

9:40am **BI-TuM5 Surfaces Engineered to Target Integrins to Direct Cell Adhesion and Function**, ***A.J. Garcia***, Georgia Institute of Technology **INVITED**

Cell adhesion to adsorbed extracellular matrix proteins and adhesive sequences engineered on synthetic surfaces plays a critical role in numerous biomaterial, tissue engineering, and biotechnological applications. Cell adhesion to adhesive motifs is primarily mediated by integrin adhesion receptors. In addition to anchoring cells, supporting cell spreading and migration, integrins provide signals that direct cell survival, proliferation, and differentiation. We have developed two biomolecular strategies for the engineering of surfaces to control integrin binding and cell adhesion in order to direct cell function. The first approach focuses on surfaces presenting well-defined chemistries that control protein adsorption to modulate integrin binding in order to potentiate cell adhesion and signaling thereby directing cell differentiation. In a second approach, we have engineered fibronectin- and collagen-mimetic surfaces presenting controlled ligand densities in a non-fouling background that promote the binding of specific integrin receptors and direct adhesive interactions. These surface engineering strategies provide a basis for the rational design of robust biospecific surfaces that tailor adhesive interactions and elicit specific cellular responses for the development of bioactive implant surfaces, 3D hybrid scaffolds for enhanced tissue reconstruction, and growth supports for enhanced cellular activities.

10:20am **BI-TuM7 Atomic Force Microscopy Imaging and Force Spectroscopy of Microbial Cell Surfaces**, ***Y.F. Dufrene***, Université Catholique de Louvain, Belgium **INVITED**

The advent of atomic force microscopy (AFM) has recently opened a wide range of novel possibilities for probing microbial cell surfaces on the nanoscale.@footnote 1@ Using AFM imaging in aqueous solution, microscopists can visualize cell surface nanostructures (surface layers, appendages), follow physiological changes (germination, growth) and monitor the effect of external agents (antibiotics, metals) in real-time.@footnote 2@ Further, using force spectroscopy, researchers can learn about local biomolecular interactions and physical properties. Spatially resolved force mapping offers a means to determine physical/chemical heterogeneities at the subcellular level, thereby providing complementary information to classical characterization methods. Force measurements allow the cell surface elasticity to be determined.@footnote 3@ Functionalizing the AFM tip with chemical groups or biomolecules enables quantitative measurements of surface charge,@footnote 4@ surface hydrophobicity@footnote 5@ and receptor-ligand interactions. Finally, single-molecule force spectroscopy can be applied to cell surface molecules to gain insight into their mechanical

properties.@footnote 6@ Clearly, these new AFM-based experiments contribute to improve our understanding of the structure-function relationships of microbial cell surfaces and will have considerable impact on biotechnology and medicine. @FootnoteText@@footnote 1@Y.F. Dufrene, J. Bacteriol., 184, 2002, 5205-5213. @footnote 2@Y.F. Dufrene, C.J.P. Boonaert, P.A. Gerin, M. Asther, P.G. Rouxhet, J. Bacteriol., 181, 1999, 5350-5354. @footnote 3@H.C. van der Mei, H.J. Busscher, R. Bos, J. de Vries, C.J.P. Boonaert, Y.F. Dufrene, Biophys. J., 78, 2000, 2668-2674. @footnote 4@F. Ahimou, F.A. Denis, A. Touhami, Y.F. Dufrene, Langmuir, 18, 2002, 9937-9941. @footnote 5@Y.F. Dufrene, Biophys. J., 78, 2000, 3286-3291. @footnote 6@B.C. van der Aa, R.M. Michel, M. Asther, M.T. Zamora, P.G. Rouxhet, Y.F. Dufrene, Langmuir, 17, 2001, 3116-3119.

11:00am **BI-TuM9 Molecule Specific Imaging Analysis of Carcinogens in Breast Cancer Cells using Time-of-Flight Secondary Ion Mass Spectrometry**, ***K.J. Wu***, *J.N. Quong, M.G. Knize, K.S. Kulp,* Lawrence Livermore National Laboratory

The concentration and localization of intracellular chemical compounds such as pharmaceuticals and carcinogens are important, specifically for application in physiology and medicine. Cooked muscle meats contain small amounts of rodent carcinogens belonging to the heterocyclic amine class of compounds and are implicated in human cancers at various organ sites. 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is the most mass abundant in cooked foods. PhIP is a genotoxic carcinogen, which has been demonstrated to cause dose-dependent mammary and prostate tumor formation in rats. In this report we will present the results on time-of-flight secondary ion mass spectrometry (TOF-SIMS) studies of several MCF7 line of human breast cancer cells. Protocols for high vacuum compatible tissue and cell culture preparation have been developed. Such direct imaging approach permits an acquisition of element and molecule-specific images directly from the cell surface. The results show the intracellular concentration and distribution of low level carcinogenic compounds such as PhIP in flash-frozen MCF7 cells; the time dependent effects of heterocyclic amine carcinogens interaction with MCF7 cells. We will discuss two major efforts to further the imaging mass spectrometry applications for biological samples: ME-SIMS approach to enhance the ionization yields and multivariate analysis data reduction technique for compound distribution on cell surfaces. @FootnoteText@ This work was performed under the auspices of the U.S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48.

11:20am **BI-TuM10 Preparation and Characterization of Chemically Patterned Surfaces for Cell-Surface Interaction Studies**, ***D. Marton***, *E.A. Sprague,* The University of Texas Health Science Center at San Antonio*; K. Cho,* University of Michigan Medical Center*; J.C. Palmaz,* The University of Texas Health Science Center at San Antonio

There is significant evidence that surface chemistry plays a major role in the way proteins and living cells interact with biomaterial surfaces. These phenomena could be best studied on surfaces with designed and identifiable chemically different areas. For this purpose we developed surface patterning techniques that combine vacuum deposition (dc sputtering or evaporation) and sputter removal of material. Four types of patterned surfaces were developed. Substrates were made of either pre-treated hydrophilic silicon or hydrophobic Teflon. Two types of specimens were created using each substrate type. The first set of patterned surfaces, called "dot pattern" comprised of carbon, stainless steel and gold dots of nominal 25 micron diameter directly deposited on the clean substrates through a stainless steel mask with a polka-dot type hole arrangement. The second set of patterned surfaces, called "hole pattern" were produced by first depositing a continuous layer of carbon, stainless steel or gold. The specimens were then sputtered through the holes of the mask using 1-2 keV Ar ions until the underlying Teflon or silicon became exposed. Typical film thicknesses were 5-10 nm. All specimens were analyzed to verify the patterning on five areas using ToF-SIMS imaging and some using XPS imaging. Pattern definition depends on mask apposition, and, in the case of insulating specimens, on charging effects. In general, the hole patterns have sharper boundaries than the dots. Using an in vitro cell migration model, human aortic endothelial cells were observed to respond to the different patterns with respect to cell shape and cell migration rate. Pattern dependent protein adherence was also observed.

11:40am **BI-TuM11 Drug Testing and Environmental Toxin Detection using Cell-based Biosensors**, ***A. Natarajan***, *P. Molnar, K. Sieverdes, A. Jamshidi, J.J. Hickman,* Clemson University

In the last decade the threat of environmental pollution, biological warfare and new diseases has increased research into cell-based biosensors. The need for more advanced methods to evaluate candidates has pushed this area of research even further. Cells are natural sensors in the body and react to different bioactive compounds in specific ways. Our research exploits this feature, using the ion channels of electrically active cells like cardiac myocytes, to create a database of specific responses. These responses can then be used to detect the acute presence of a substance. A traditional method of studying drug and toxin effects has been low throughput patchclamp electrophysiology. Our cell-based biosensor consists of a uniform monolayer of cardiac myocytes on a microelectrode array (MEA) with 60 substrate-integrated electrodes. The microelectrode arrays are surface modified and the media used for the cells is defined. Surface analysis was used to verify surface modifications. Long-term recordings of beating cells produced extracellular field potentials in the range 100 µV to 1200 µV, with a beating frequency of 0.5 to 4 Hz. The toxins tested were classified as drugs (epinephrine), heavy metals (Cadmium) and pyrethroids, a group of synthetic pesticides. Pyrethroids modify sodium channels thus disrupting nerve cells in insects. Epinephrine is a well-known stimulant for the heart. Cadmium chloride causes serious illnesses with the same symptoms as lead poisoning. Concentrations used were mainly 1, 10 and 100 µM. Each of the above substances produced specific responses in the action potential signals, changing amplitude, frequency and shape. 10 µM Epinephrine increased spike frequency from 4 Hz to 6 Hz. Our goal is to replace patchclamp electrophysiology with microelectrode arrays as a means to testing many drugs and toxins. Future work involves making this system more stable, creating a larger database and extending the use for chronic detection of compounds.

Applied Surface Science
Room 324/325 - Session AS+BI-TuA

Biomaterials Characterization

Moderator: J.E. Fulghum, University of New Mexico

2:00pm **AS+BI-TuA1 Spatially Defined Immobilization of Biomolecules on Microstructured Polymer Substrate***, **A. Hozumi**, N. Shirahata,* National Institute of Advanced Industrial Science and Technology, Japan*; S. Asakura, A. Fuwa,* Waseda University, Japan*; Y. Yokogawa, T. Kameyama,* National Institute of Advanced Industrial Science and Technology, Japan

ã€€The spatial arrangement of biomolecules on solid surfaces with artificial control in the micro~nanometer scale has attracted attention in biotechnical and biomedical applications. Here we report a simple method by which a number of biomolecules can be immobilized onto positions spatially defined in micrometer-scale. Our approach demonstrated here is based on the photodecomposition and hydrophilization of polymeric material using vacuum ultraviolet (VUV) light of 172 nm radiated from a Xe@sub 2@@super *@ excimer lamp. Each poly (methyl methacrylate) (PMMA) substrate was irradiated for 30 min at 10@super 3@ Pa with VUV light through a photomask contacting the PMMA surface. As confirmed by atomic force microscopy, after VUV-irradiation, microwell arrays composed of about 2 nm in diameter and 350 nm in depth were successfully formed on the PMMA substrates. Next, using such microstructured PMMA substrates, we demonstrated spatial arrangement of biomolecules. The microstructured sample was immersed into a solution containing antibodies labeled with fluorescence for 30 min. The antibodies were selectively adsorbed on the microwells in which the surfaces were photooxidized, while the surrounding regions where they were not unirradiated regions remained free of adsorption, as evidenced by fluorescence microscopy. This specific adsorption was probably due to the differences in chemical properties between the VUV-irradiated and unirradiated regions, as well as due to the geometrically effect. Indeed, according to water-contact angle measurements and X-ray photoelectron spectroscopy analysis, the VUV-irradiated PMMA surface became highly hydrophilic with its water-contact angle changing from 80Ã…Ã£ to 25Ã…Ã£ due to the formation of polar-functional groups, such as C=O and O-C=O, on the surface. Such chemically and geometrically defined microwells are expected to serve as spatially arranged active sites for the immobilization of a wide variety of biomolecules.).

2:40pm **AS+BI-TuA3 Low-Temperature STM Manipulation of Single Bio Molecules***, **J.J. Benson**, V. Iancu, A. Deshpande, S.-W. Hla,* Ohio University, Athens

Single porphyrin molecules adsorbed on Cu(111) surface are investigated by using a variety of manipulation procedures and spectro/microscopy measurements with a low temperature UHV STM at 6 K. The tunneling I/V and dI/dV spectroscopy techniques are used to probe the electronic properties of the single molecules with atomic level precision.@footnote 1@ Mechanical stability of single molecules is also examined using 'lateral manipulation' techniques with the STM-tip.@footnote 2@ In this procedure, the STM-tip is brought very close to the molecule to increase tip-molecule interactions (approximately one angstrom from the molecule). Then the tip is moved across the surface. Due to the tip-molecule interaction, the molecule is pushed across the substrate to relocate it to specific surface sites. Detailed internal conformation changes of the molecule can be directly monitored through the corresponding STM-tip height signals during the lateral manipulation process. These combined STM manipulation/spectroscopy investigations elucidate detailed information about the electronics and mechanical properties of the porphyrin molecules at sub-nanometer level resolutions. @FootnoteText@@footnote 1@F. Moresco et al, Phys. Rev. Lett. 86, 672-675, (2001). @footnote @@S.-W. Hla, K.-H. Rieder, Ann. Rev. Phys. Chem. 54, 307-330, (2003). .

3:00pm **AS+BI-TuA4 Base-dependent Displacement of Thiolated DNA Films by Mercaptohexanol (MCH)***, **H. Kimura-Suda**,* National Institute of Standards and Technology*; D.Y. Petrovykh,* University of Maryland & Naval Research Laboratory*; L.J. Whitman,* Naval Research Laboratory*; M. Tarlov,* National Institute of Standards and Technology

The immobilization of DNA on surfaces is the basis for DNA microarrays and many emerging nanotechnology applications. It has been demonstrated that the attachment of thiolated DNA probes to gold surfaces is an effective approach for construction of DNA-based sensors and diagnostics. One challenge with the use of thiolated DNA is reproducibly controlling the surface coverage and hybridization activity of adsorbed probes. A two-step method, where first the gold substrate is exposed to a solution of thiol-modified single-stranded DNA (HS-DNA), followed by exposure to a solution of mercaptohexanol (MCH), is a common approach for controlling the coverage and orientation of DNA probes. In this protocol, MCH both passivates the surface against nonspecific adsorption of DNA targets and "activates" DNA probes by displacing adsorbed nucleotides from the gold surface. The MCH treatment also displaces DNA probes from the gold surface resulting in less steric hindrance for hybridization. Nonetheless, the displacement of thiolated DNA by MCH remains poorly understood. In this study, we focused on base-dependent displacement of HS-DNA films from gold upon MCH exposure. Self-assembled monolayers of thiolated homo-oligonucleotides [HS-(dA), HS-(dT), HS-(dC), HS-(dG)] on gold surfaces were produced and characterized before and after exposure to MCH with FTIR and XPS. Surprisingly, we find that the displacement of HS-DNA on gold by MCH is strongly base-dependent. For example, most HS-(dT) is removed or displaced, whereas most HS-(dC) remains on the surface. In this talk we will present a selectivity series for the base dependent displacement of homo-oligonucleotides by MCH and discuss the origin of this effect. We will demonstrate that base dependent displacement effects can account for dramatic variations in probe coverage for probes of different base composition.

3:20pm **AS+BI-TuA5 Neuron Pathfinding and Surface Chemistry, Patterning and Reactions***, **T.P. Beebe, Jr.**,* University of Delaware **INVITED**

Biomaterials interfaces are at the heart of new approaches to control cell-surface interactions, and modern surface analytical techniques can now provide molecular-scale information about surface modifications, coverages and patterning or relevant ligands and proteins. These approaches can inform our understanding of the relationship between surface chemistry, surface structure and biological function. Using the biomedical problem of repair to damaged central nervous system tissue as the motivation for biomaterials interface characterization and cell-surface interactions, we will present several approaches to surface modification and surface characterization in conjunction with cell-surface biophysical measurements. The tools for these studies are AFM, XPS, TOF-SIMS and fluorescence microscopy and labeling.

4:00pm **AS+BI-TuA7 In-situ Spectroscopic Study of Thermal Phase Transition of Supported Hybrid Bilayer Membranes***, **C.S.-C. Yang**, K.A. Briggman, J.C. Stephenson, L.J. Richter,* National Institute of Standards and Technology

Hydrated phospholipid structures (Langmuir-Blodget films, supported bilayers, vesicles, etc.) have been widely studied as model systems for biological membranes. We report a study of the thermal phase transitions of fully hydrated hybrid bilayer membranes, i.e. phospholipid monolayers self-assembled onto a Au surface previously modified by a self-assembled monolayer of octadecane thiol (ODT). Using Sum Frequency Generation, a non-destructive interface-sensitive nonlinear optical probe, the structure and conformation of both the ODT and phospholipid alkyl chains have been characterized as a function of temperature from 25 to 60 °C. There is very little change in the ODT alkyl chain order over the temperature range studied. There are significant changes in the lipid chain order that are attributed to the transition from the ordered gel phase to disordered fluid phase, allowing us to determine the phase transition temperatures of the two-dimensional lipid layer. The gel-fluid phase transitions for a series of saturated phospholipids in the hybrid bilayers are observed at ~ 10 °C higher temperatures than those in corresponding multilamellar vesicles.

4:20pm **AS+BI-TuA8 Spectroscopic Quantification of Covalently Immobilized Oligonucleotides***, **A.V. Saprigin**, C.W. Thomas, C.H. Patterson, M.S. Spector,* Naval Research Laboratory

Quantitative determination of surface coverage, film thickness, and molecular orientation of DNA oligomers covalently attached to aminosilane monolayers has been obtained using complementary infrared and photoelectron studies. Spectral variations between the different nucleic acids are observed in surface immobilized oligomers for the first time. Carbodiimide condensation was used to covalently attach phosphorylated oligonucleotides to silanized aluminum substrates. Fourier-transform infrared (FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS) were used to characterize the surfaces after each modification step. Infrared reflection-absorption spectroscopy of covalently bound DNA provides orientational information. Surface density and layer thickness are extracted from XPS data. The surface density of immobilized DNA, 2-3Ã—10^13 molecules/cm^2, was found to depend on base composition. Comparison of antisymmetric to symmetric phosphate stretching band intensities in reflection-absorption spectra of immobilized DNA and

transmission FTIR spectra of DNA in KBr pellet indicates that the sugar-phosphate backbone is predominantly oriented with the sugar-phosphate backbone lying parallel to the surface, in agreement with the 10-20 Ã… DNA film thickness derived from XPS intensities.

4:40pm **AS+BI-TuA9 Photoionization for Trace Measurement of DNA on Surfaces**, ***J.F. Moore**, W.F. Calaway,* Argonne National Laboratory*; B.V. King,* University of Newcastle, Australia*; J.W. Lewellen, S.V. Milton, M.J. Pellin,* Argonne National Laboratory*; M. Petravic,* Australian National University*; I.V. Veryovkin,* Argonne National Laboratory*; G.L. Woloschak,* Northwestern University

Recent developments in vacuum ultraviolet (VUV) lasers allow new photoionization techniques to be applied to surface and interface analysis problems. Single photon ionization of laser desorbed nucleosides and DNA was performed using a molecular F@SUB 2@ laser (wavelength 157 nm, pulse energy 8 mJ, pulse length 10 ns) and a tunable free electron laser (wavelength 120 - 265 nm, pulse energy 0.1 mJ, pulse length 300 fs). Results including detection limit and degree of fragmentation are compared for several systems including guanosine and single-stranded DNA of 10-30 base pair lengths. The tunability of the free electron laser to a wavelength just above the ionization potential of the analyte molecule can be used to enhance selectivity and sensitivity of the analysis. There are clear applications of this sensitive, selective, spatially resolving technique that is capable of identifying mutated or adducted DNA with little sample preparation. These uses will be elaborated on in the context of our results and plans for further technique development, and operational experience with the free electron laser. @FootnoteText@ This work is supported by the U. S. Department of Energy, BES-Materials Sciences, under Contract W-31-109-ENG-38.

5:00pm **AS+BI-TuA10 Utilization of Polyatomic Primary Ion Sources for Analysis of Drug Delivery Systems by Secondary Ion Mass Spectrometry (SIMS)**, ***C.M. Mahoney**, G. Gillen,* National Institute of Standards and Technology

The utilization of cluster primary ion beams in SIMS has become very popular in the last decade due to the increased secondary ion yields as compared to monoatomic sources.@footnote 1-4@ In particular the analysis of organic materials has gained considerable interest as these cluster primary ion beam sources (in particular SF@sub 5@@super +@) have resulted in the enhancement of characteristic molecular secondary ion yields and have decreased the beam induced damage.@footnote 4@ Furthermore, the increased sputter rate with decreased beam damage has allowed for depth profiling in organic films and polymers for the first time with limited success.@footnote 4@ Here we explore the applicability of cluster SIMS in the analysis of various materials utilized in drug delivery. The effects of SF@sub 5@@super +@ bombardment on molecular secondary ion yields will be explored in various biodegradable polymers (polylactic acid, polyglycolic acid and polycaprolactone) as well as several model drugs (theophylline, 4'-hydroxyacetanilide, amyloid probe). The enhancement in the sensitivity will then be applied to imaging applications where it will be shown that imaging with SF@sub 5@@super +@ enhances the signal intensity as compared to Ar or Cs primary ions resulting in more sensative imaging capabilities. This will be useful in many systems where the concentration of drug is very low (e.g. biological samples, ppb-ppm range). Dynamic SIMS analysis (utilizing SF@sub 5@@super +@) of a series of polylactic acid films doped with varying concentrations of 4'-hydroxyacetanilide will also be discussed. @FootnoteText@ @footnote 1@ Kotter, F.; Benninghoven, A. Applied Surface Science 133 (1998) 47.@footnote 2@ Appelhans, A.D.; Delmore, J. Anal. Chem. 61 (1989) 1087.@footnote 3@ Gillen, G.; King, R.L.; Chmara, F. J. Vac. Sci. Technol. A 17(3) (1999) 845.@footnote 4@ Gillen, G.; Roberson, S.; Rapid Commun. Mass Spectrom. 12 (1998) 1303.

Biomaterial Interfaces

Room Hall A-C - Session BI-TuP

Poster Session

BI-TuP2 Plasma Processing pf Polymers to Reduce Bio-Fouling for Cardiac Applications, ***M. Neumann***, *P. Fackler, D.N. Ruzic,* University of Illinois at Urbana-Champaign

Polymers are playing an increasing role in cardiac medicine as components of implants and diagnostic devices, such as tubes, diaphragms, filters, pacemaker components, blood bags, sutures, vascular grafts and shunts. Polymers exhibit high strength to weight ratio, wide range of flexibility, ease of formability, and economics of production, but processes designed to achieve desired surface properties can compromise the overall bulk material. The ability to alter the surface of the polymer while leaving the underlying bulk material unchanged has a large potential for development in the area of biomaterials. Modifying the surface of a polymeric material so as to impede water adhesion and the ability of proteins, bacteria, and cells to grow on the surface can be minimized on those surfaces that are incorporated into biological systems. This can minimize infection, thrombosis, and other undesirable interactions. Polymer surface modification was accomplished via plasma etching and deposition in a commercial size plasma-etching device, which achieves plasma densities and electron temperatures up to 10@super 11@ cm@super -3@ and 4 eV. The degree of change is controlled by macroscopic external controls, rather than invasive internal modifications. This process lends itself well for use in existing systems. Water contact measurements taken before and after treatment of HDPE that show a change from a pretreatment angle of 85° to post treatment angles of 5°, which corresponds to a dramatic change in surface energy of the polymer and biological interaction potential. This modification is both physical and chemical, but limited to a few microns of the surface of the material. Plasma analysis is done through use of Langmuir probes, microwave interferometery, and optical spectroscopy. Surface analysis is done through XPS. Blood-material interactions are studied through two-dimensional electrophoresis in order to determine the extent and nature of protein coverage.

BI-TuP3 Non-Fouling Surfaces for Biosensors and Biomaterials, ***L. Li***, *S. Chen, J. Zheng, S. Jiang,* University of Washington

Non-fouling surfaces are critical to the performance of biosensors and biomaterials. Despite of their importance and enormous effort, non-fouling mechanism is still not quite clear at present. It was shown in our previous work that the behavior of protein adsorption depended on nano-scale structures of a surface with which proteins interact. Therefore, molecular details of a surface are of great importance to protein adsorption. However, there is still a considerable lack of the fundamental understanding of how nano-scale structures affect protein adsorption at the molecular level. The objective of this work is to demonstrate that nano-scale structures of a surface are responsible for protein resistance. Polyethylene glycol (PEG) self-assembled monolayers (SAMs) are used as model systems and the nano-scale structures of the surfaces are altered by adjusting factors such as the assembly substrates, the assembly conditions, and the composition of mixed SAMs. These SAMs are characterized by atomic force microscopy (AFM)/scanning tunneling microscopy (STM) and X-ray photoelectron spectroscopy (XPS). Protein adsorption on these surfaces was investigated by surface plasmon resonance (SPR) biosensors and tapping-mode AFM. Furthermore, experimental results are directly compared with those from molecular simulations, which can also provide additional information not easily accessible to current laboratory experiments, such as adsorbed water structures at protein/SAM interfaces. Results from combined experimental and simulation studies provide insight on how nano-scale structures affect protein adsorption and shed light on non-fouling mechanisms.

BI-TuP4 Patterned Immobilization of Proteins on High-density PEG Coated Si Surfaces, ***Y. Jun***, *X.-Y. Zhu,* University of Minnesota

Spatially localized patterns of chemical and biological functions on solid surfaces are of great interest to a number of research fields, such as biochips and cell biology. We have developed a novel chemical strategy for the formation of micro-scale patterns of chemical and biological functions on silicon surfaces using soft lithography techniques. This is based on an efficient reaction between alcohol functional groups and chlorine terminated silicon surfaces. The features formed by soft lithography show exceptionally high resolution and fidelity, with edge resolution as high as 10 nm. We demonstrate the immobilization of protein molecules to spatially localized surface functional groups on high-density PEG brush coated silicon. The spatially localized activation of the PEG brush is achieved via either partial oxidation to form aldehyde groups or via attachment of efficient leaving groups. Protein molecules are covalently immobilized to these activated regions on the PEG/Si surface.

BI-TuP5 The Topography and Viscoelastic Properties of Salivary Pellicle and Their Interaction with Tannins, ***M.E. Dickinson***, *A.B. Mann,* Rutgers University

The acquired pellicle is an organic film formed by the adsorption of proteins and carbohydrates on dental enamel exposed to the oral environment. The pellicle is now understood to have several roles including lubrication and reduction of friction between teeth during mastication, as well as protection against acidic solutions. Using in vitro AFM, growth, structure and topology of pellicle grown in vivo on a clean enamel surface has been studied. Complete coverage of the enamel surface was found to occur within the first few minutes of exposure, with the layer increasing in thickness until a maximum is reached at around 2 hours. The thickness of the pellicle varies with position, but fully developed pellicle can range in thickness from 200-500nm. Tannins found in food can interact with pellicle to change its viscoelastic properties and alter the color which is a cause of extrinsic staining in teeth. Tannins are phenolic compounds capable of precipitating alkaloids, gelatin and other proteins, they are naturally found in many food stuffs especially leafy products such as tea. The topology of the pellicle surface, as studied using AFM and SEM, consists of a dense arrangement of adsorbed globular shaped proteins with no break in the structure, even when tannins are added. The viscoelastic properties of the pellicle have been studied using nanoscale dynamic mechanical analysis, this shows that substantial variations in the storage and loss modulus occur with increasing exposure to tannin containing solutions. These changes in viscoelasticity will impinge directly on the pellicle's performance as a lubricant and also its ability to act as a chemical barrier to acid attack.

BI-TuP7 Attachment of Blood Proteins to Chitosan Surfaces, ***D.W. Thompsons***, *W.H. Nosal, S. Sarkar, A. Subramanian, J.A. Woollam,* University of Nebraska, Lincoln

Chitosan is a biomaterial of interest because of the potential to modify its amine groups to control its interactions with surfaces. Though chitosan itself is thrombogenic, such modifications may lead to a surface with enhanced biocompatibility. In this work we use spectroscopic ellipsometry to monitor the attachment of three blood proteins (albumin, IgG, and fibrinogen) to a spin-cast chitosan surface in phosphate-buffered saline solution. The moisture-induced swelling of the chitosan film is characterized, and optical constants for chitosan and the proteins are determined independently for use in modelling the time-dependent data. Ex-situ infrared ellipsometry is used to characterize the chemical nature of the protein attachment and compare chitosan films with varying degrees of deacetylation. Material anisotropy due to preferred molecule orientation is apparent from ellipsometry.

BI-TuP8 Operation of the QCM-D Technique at Elevated Oscillation Amplitudes, ***M.E.M. Edvardsson***, *F. Höök,* Chalmers University of Technology, Sweden

An often raised question with respect to applications of the quartz crystal microbalance technique is whether the shear oscillation of the sensor surface influences the measured binding events. This is indeed a relevant question, especially since solid proofs for bond rupture at elevated amplitudes was recently presented.@footnote 1,2@ In part inspired by these observations, our QCM-D device has been further developed to operate at variable driving amplitudes (from 1 V to 10 V corresponding to maximum oscillation amplitude between 4nm and 40nm), while still maintaining the possibility to perform combined f and D measurements - a combination that was not implemented in previous work. In order to test the device, we choose the well established process by which intact vesicles adsorb and decompose into a planar supported lipid bilayer on SiO@sub 2@, known to be very sensitive to external perturbations. Up to driving amplitudes of 2V, the adsorption behavior is essentially unaffected, whereas it becomes significantly affected at driving amplitudes above 2V. These results thus nicely demonstrate the possibility to implement variable driving amplitudes, and proves that an entirely new dimension will be possible to explore in detail, especially when combined with f and D data at multiple harmonics and proper theoretical modeling is implemented: The amplitude variation can be used to either affect the system being studied, or to extract new information about it by going from the harmonic to the anharmonic regime, which will be discussed in this paper. @FootnoteText@ @footnote 1@Cooper MA, et al.; Direct and sensitive detection of a human virus by rupture event scanning. Nature

Biotechnology 2001, 19:833-837@footnote 2@Dultsev FN, et al.; "Hearing" bond breakage. Measurement of bond rupture forces using a quartz crystal microbalance. Langmuir 2000, 16:5036-5040.

BI-TuP9 Protein Immobilization for Multi-Channel Sensor Detection, ***J. Ladd***, *Q. Yu, S. Chen,* University of Washington*; J. Homola,* Institute of Radio Engineering and Electronics, Czech Republic*; S. Jiang,* University of Washington

The simultaneous detection of multiple analytes is an important consideration for the advancement of current biosensor technology. Currently, few sensor systems possess the capability to accurately and precisely detect multiple antigens. The work presented demonstrates a novel approach for the functionalization of sensor surfaces for multi-channel detection. This approach combines inkjet-printing technology with self-assembled monolayer (SAM) chemistry to create a protein array. A modified commercial Epson C40UX inkjet printer is used in this work. The sensor platform is based on a layer of streptavidin immobilized on a mixed SAM of biotinylated alkanethiol (BAT) and poly(ethylene oxide) (PEO). Non-specific binding, and thus false positives, are minimized with the non-fouling background of the sensor surface. The described platform is used in a home-built surface plasmon resonance (SPR) biosensor. Results show excellent specificity in protein immobilization to the proper locations in the array, eliminating the possibility of a false detection within a channel. Analysis of multiple proteins in solution shows a similar behavior and response to pure protein solutions. The detection capabilities of a sensor using this protein array have been characterized using human choriogonadotropin (hCG).

BI-TuP10 Chemisorption of Aromatic Amino Acid Derivatives on Gold Surface, ***R.M. Petoral, Jr.***, *K. Uvdal,* Linköping University, Sweden

The interfacial property of adsorbate and thin layers of biomolecules on solid surfaces is of great significance in biomaterials and biosensor application. Understanding the binding and molecular orientation of the adsorbates is then of great importance. Amino acids with aromatic side chains such as Tyrosine and 3,4-dihydroxyphenylalanine (DOPA) is linked to a short thiol through a peptide bond and is adsorbed and self-assembled to polycrystalline gold surfaces. The molecular adsorption, chemical binding and orientation of the amino acid analogue to the surface are studied by X-ray Photoelectron Spectroscopy (XPS), Infrared Reflection-Absorption Spectroscopy (IRAS) and Near-edge X-ray Absorption Fine Structures (NEXAFS). Strong molecular binding of the amino acid derivatives on gold surface through the sulfur atom was attained. Angle dependent XPS results showed that the aromatic ring is oriented away from the gold surface. Parallel orientation of the C=O bond of the amide moiety relative to the gold surface is deduced from the IRAS and NEXAFS results. The average orientation of the aromatic ring and main molecular axis of the molecules relative to the gold surface are also determined. The aromatic amino acid derivatives are able to self-assemble and form an ordered monolayer with minimal degree of orientational disorder. Results from this experiment are valuable in our development of sensor surfaces to be used for interaction studies with other biomolecules and metal ions.

BI-TuP11 Multi-Technique Characterization of Self-Assembled Peptide Monolayers, ***N.T. Samuel***, University of Washington*; K. McCrea,* Polymer Technology Group*; L.J. Gamble,* University of Washington*; D.A. Fischer,* National Institute of Standards and Technology*; P.S. Stayton,* University of Washington*; G.A. Somorjai,* University of California, Berkeley*; D.G. Castner,* University of Washington

There is considerable interest in the immobilization of bioactive peptides for applications such as affinity separations, diagnostics, cell culture and biomedical implants. We have synthesized a series of lysine and leucine containing peptides, which are designed to attach to surfaces with different secondary structures (alpha helix, beta sheet, etc.). These peptides were attached to carboxy-terminated self-assembled monolayers and characterized with ToF-SIMS (Time-of-Flight Secondary Ion Mass Spectrometry), SFG (Sum Frequency Generation) and NEXAFS (Near Edge X-ray Absorption Fine Structure Spectroscopy). The ToF-SIMS spectra from these peptides were analyzed by principal component analysis. Principal component 1 (PC 1), which captures 89% of the variance in the spectrum, represents the variation in the bulk amino acid composition of the different monolayers. PC 2, which captures 10% of the variance, separates the peptides with different secondary structures, suggesting ToF-SIMS is sensitive to different secondary structures of the peptides. SFG spectra were acquired for the alpha helical peptide adsorbed onto both hydrophobic and negatively charged substrates. The hydrophobic surface spectrum showed strong peaks in the CH stretch regions while the negatively charged surface spectrum showed strong peaks in the NH stretch region. The SFG results indicate the alpha helical peptide binds differently to these two surfaces. These observations were also confirmed by ToF-SIMS experiments, which reveal a strong dependence of the amount of peptide adsorbed onto negatively charged substrates when the solution pH is varied. We have recently developed a simple protocol to attach a short thiol linker onto these peptides at their amine terminus. This provides an additional handle to immobilize these peptides onto surfaces and control their orientation. Polarization-dependent NEXAFS experiments on these monolayers are in progress.

BI-TuP12 Molecular Simulation of Cytochrome C Adsorbed on Self-Assembled Monolayers, ***J. Zhou***, *J. Zheng, S. Jiang,* University of Washington

Cytochrome c, a membrane electron transfer protein, plays an important role not only in a wide range of basic life processes, but also in biomaterial and biosensor applications. To enable the electron transfer fast, cytochrome c should sit on the surfaces with an orientation that the heme ring close and perpendicular to surfaces. Moreover, the adsorbed cytochrome c should keep its native conformation. Self-assembled monolayers are ideal platforms for the study of protein adsorption. In this work, the orientation and conformation of cytochrome c on charged self-assembled monolayers are investigated by a combined Monte Carlo and molecular dynamics simulation approach. The effects of positively and negatively charged SAMs, degree of dissociation of COOH-terminated SAM, ionic strength are examined. CHARMM force field was used to model the protein and SAM. Both implicit and explicit solvent model were used. The root mean square deviation, gyration radius, eccentricity, Ramachandran angles, heme orientation and superimposed structures of cytochrome c were calculated during the simulation. Simulation results show that desired orientation could be obtained on a negatively charged surface. The dissociation degree of the terminal group affects the conformation of adsorbed protein. This work sheds light on the mechanism of the orientation and conformation of adsorbed proteins at the molecular level, and would be useful for the design and development of biosensors and biomaterials.

BI-TuP13 SPARC Binding with ECM Proteins and its Influence on Cell Adhesion, ***H. Wang***, *S. Chen,* University of Washington*; T. Barker, H. Sage,* Hope Heart Institute*; B.D. Ratner, S. Jiang,* University of Washington

The secreted protein acidic and rich in cystein (SPARC/osteonectin/BM-40) is associated with events characterized by changes in cell shape and mobility. Although the molecular mechanism remains unclear, it is generally believed that the counter-adhesive properties of SPARC are related to its interactions with ECM proteins. In this study, the interactions of SPARC with ECM proteins, such as collagen I and fibronectin, are characterized and quantified using atomic force microscope (AFM) and surface plasma resonance (SPR), and their influence on cell adhesion are examined. AFM can characterize the binding of SPARC with both collagen I and fibronectin at the molecular level. SPARC has been shown to interact with collagen I, but direct topographic image has not been reported. Whether SPARC interacts with fibronectin still remains inconclusive. In this work, AFM is used to determine the number and location of binding sites on individual collagen I and fibronectin. Monoclonal antibodies of SPARC are used to assist for better visualization. These studies provide direct information about how and where binding occurs. SPR is used to quantify these interactions. It was shown that these interactions have ionic nature. Furthermore, the C-terminal region of SPARC, which contains a high-affinity Ca2+-binding site, may play an important role in its binding with ECM proteins. Thus, the influence of ionic strength and concentration of Ca2+ on binding are studied in SPR experiments. Cell culture and adhesion assays are used to study SPARC as a modulator of the adhesive process of cells seeded on ECM proteins. The influence of SPARC-collagen I interaction is studied using smooth muscle cells while the influence of SPARC-fibronectin interaction is studied using endothelial cells. The number and spreading area of cells, as well as the focal adhesion plagues are obtained as a function of the relative amount of SPARC added.

BI-TuP14 Molecular Dynamics Study of Protein Adsorption on Controlled Surfaces, ***J. Zheng***, *S. Jiang,* University of Washington

Surface resistance to protein adsorption is currently a subject of great interest with potential applications in many areas, including biomaterials and biosensors. Despite its importance, there has been a lack of molecular-level understanding of protein interactions with surfaces and the mechanism of resistance to protein adsorption remains a problem to be solved. It is well known that SAMs presenting oligo (ethylene glycol) (OEG)

groups, such as S(CH2)m-(EG)nOH or -(EG)nOCH3 resist the adsorption of proteins. The molecular-level understanding of protein resistance to surfaces is needed in order to provide insights of non-fouling mechanism. In the work, we investigate interactions of protein (e.g., lysozyme) with OEG SAM (inner) surfaces using a combined Monte Carlo and molecular dynamics simulation approach in the presence of explicit water molecules. The CHARMM (version 27), an all-atom potential force field, was used to model the protein and methyl terminated SAMs. The TIP3P potential was used to describe water interactions. For the OEG terminated SAMs, we used a SJY model that reproduces very well the helix structure of OEG tails. Results from inert surfaces will be compared with those from methyl terminated SAM surfaces. The behavior of water at protein/SAM interfaces is characterized by self-diffusion coefficient, order parameter, hydrogen bonding, and radial distribution. In these simulations the effects of surface (charge, hydrophobicity, and defect), solvent, pH, and ion strength will be taken into account. Results from this work will shed a light on non-fouling mechanism at the atomic-scale level and guide the design of better biocompatible materials and biosensors.

BI-TuP15 Creation of Surface Macromolecular Docking Sites for the Reversible Immobilization of Proteins in Active Conformation and Controlled Orientation, ***G. Zhen***, *E. Zobeley, V. Eggli, R. Glockshuber, M. Textor,* Swiss Federal Institute of Technology, Switzerland

Our contribution describes the comparative performance of two docking site concepts for the immobilization of biomolecules in active conformation and controlled immobilization. They are based on biotin-(Strep)avidin and NTA-Ni@super 2+@-histag linkage techniques, in combination with polycationic, PEG-grafted, NTA(nitrilotriacetic acid) functionalized and biotinylated copolymers. Enzyme @beta@-lactamase served as the model protein for the verification of the linkage concept and for the investigation of the activity of the surface-immobilized protein and its dependence on the molecular orientation. Five different variants of @beta@-lactamase with single cysteine site-directed mutagenesis on the surface were engineered. Two platforms were tested in order to determine how the mechanical and dynamic properties of the interface influence the enzyme-orientation-dependent catalytic activity: polymeric interface (flexible chains, soft) and functionalized alkanethiol monolayers on gold (comparatively immobile, stiff). The @beta@-lactamase variants were biotinylated at free thiol-group with a cleavable biotinylation reagent allowing for controlled release of the surface-bound enzyme. The immobilization was achieved on niobium oxide surface coated with biotinylated Poly(L-lysine)-g-poly(ethylene glycol) and on gold coated with mixture of alkanethiol-biotin and alkanethiol-OH self-assembled monolayer. The biotinylated @beta@-lactamase was subsequently bound to the surface via NeutrAvidin. The long-term stability of the immobilized proteins was evaluated. The amount of immobilized @beta@-lactamase on the chips was measured by three different techniques: OWLS, SPR and specific enzymatic activity via photospectroscopic detection of the turnover of the chromogenic substrate nitrocefin. Specific immobilization could be discriminated from non-specific adsorption. Furthermore the effect of the immobilization scheme on the biological activity was quantitatively examined.

BI-TuP16 X-ray Surface Scattering for the Structural Analysis of Adsorbed Proteins at Hydrated Interfaces, ***C.A. Pavloski***, *S.S. Lateef, M.L. Schlossman, L. Hanley,* University of Illinois at Chicago

Protein adsorption onto solid surfaces is a significant process in a wide variety of applications including biomaterials, tissue engineering, biosensors, immunoassays and protein arrays. Surface properties are altered by synthetic and naturally occurring molecular adsorbates when a biomaterial is brought into contact with a biological fluid. We are interested in determining the structural conformation of adsorbed proteins at this aqueous-solid interface. We examine the surface adsorption of bovine serum albumin (BSA), the most abundant protein in blood. We bromine label BSA to allow probes of its adsorbed conformation on an amine-functionalized monolayer on a silicon wafer. We use x-ray photoelectron spectroscopy and atomic force microscopy to study the chemistry and structure of the dry surface. We then apply x-ray reflectivity and x-ray standing wave fluorescence to probe the conformation of adsorbed BSA at the hydrated interface.

BI-TuP17 Assembly and Disassembly of Hydrogels to Entrap, Grow, and Release Cells, ***G.F. Payne***, *T. Chen, L.-Q. Wu, D.A. Small, H. Yi,* University of Maryland Biotechnology Institute; *R. Ghodssi, G.W. Rubloff,* University of Maryland; *W.E. Bentley,* University of Maryland Biotechnology Institute

Hydrogels provide a bio-friendly environment for cultivating cells. Standard methods for entrapping cells within hydrogel matrices exploit the photopolymerization of synthetic monomers (or macromonomers). The strength of photopolymerization is that standard lithographic approaches can be exploited to exert considerable spatial and temporal control of hydrogel formation. The weaknesses of photopolymerization are that these methods are not always benign to cells, and the hydrogel matrices are generally permanent. We are examining an alternative method for in situ hydrogel formation based on biopolymers and enzymes. Specifically, we mix cells with the protein gelatin, and add the protein-crosslinking enzyme transglutaminase. Gel formation occurs over the course of 1-2 hours. In situ-entrapped bacterial cells (E. coli) were observed to grow to high densities within the crosslinked gelatin matrices, and these cultured cells could sense and respond to appropriate inducers (we examined the inducible expression of green fluorescent protein). After growing the cells, they could be released from the hydrogel using the protein-degrading enzyme, proteinase K. Cells were released over the course of 1 hour and they remained viable and inducible. This study demonstrates that; one enzyme (transglutaminase) can entrap cells within a hydrogel, the cells can proliferate to high densities within the matrix, and a second enzyme (proteinase K) can "dissolve" the hydrogel to release the cells. This capability should provide unique opportunities for microfluidic biosensors.

BI-TuP18 Customized Tissue Engineering Using Photopolymerizable Hydrogels and Stereolithography Techniques, ***B. Dhariwala***, *E. Hunt, T. Boland,* Clemson University

The power of tissue engineering can be enhanced using customizable scaffolds to repair defects caused by birth or accidents. For customized tissue engineering, one of the variables accessible and tunable by the engineer is the form and aspects of the scaffold onto which cells are seeded. Here we are studying hydrogels as materials that can be used for custom designed scaffolds. For this study, we employed photopolymerizable hydrogels fabricated by crosslinking polyethylene oxide (PEO) with polyethylene dimethacrylate (PEGDM) monomer using 2-methyl-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (IRGACURE) as the photoinitiator. Several hydrogels have been prepared including custom shaped hydrogels made using polypropylene molds. Initial characterization of these gels will be presented. Use of stereolithography technique has been carried out to make customized scaffolds. This is a computerized technique where a high intensity UV laser beam is used to form the hydrogel according to a 3D image of the defect. The laser scans in the X-Y plane and there is a movable platform which serves as the Z plane. Different scaffold shapes were made using this technique. Some mechanical tests were carried out on the polymer will also be overviewed and their results will be shown. Toxicity studies of the photoinitiator were carried out to determine adequate amount of initiator to be used. Initial cell studies were carried out to ensure cell viability in the polymer. Cells were mixed with the polymer, which was then photopolymerized, and their viability was studied and results will be discussed. We would further investigate cell viability & cell growth over extended periods of time. Stereolithography is a very efficient method of preparing 3D scaffolds and holds a promising future for tissue engineering.

BI-TuP19 Cell-Based Biosensors - A Tool for High Throughput Toxin Detection, ***K. Varghese***, *A. Jamshidi, K. Sieverdes, P. Molnar, M. Das, C.A. Gregory, J.J. Hickman,* Clemson University

Cell-based biosensors incorporate a cellular sensing element that detects a change in the cells immediate environment and converts the cellular signal with an electrical impulse that is conducive for integration to a silicon environment. Cell-based electrophysiology can be broadly divided into two categories - those based on intracellular potentials (eg. using glass microelectrodes as in patch clamping) and those based on extracellular potentials. Our research focuses on the latter, wherein extracellular microelectrode arrays are used as a noninvasive and long-term approach for the measurement of biopotentials. The objective of this study is to culture cardiac myocytes on Metal Microelectrode Arrays (MEAs) and Field Effect Transistors (FETs) and test them upon exposure to various toxins. The culture conditions (serum vs serum free), were also investigated to compare and contrast the results obtained therein. In the first and present part of this study, cardiac myocytes from Day 6 chicken embryos were cultured on MEAs and tested for their response to different concentrations of Cadmium Chloride and Cesium Chloride, which are known

environmental toxins. Studies also were carried out to study the effect of various adhesive surfaces on the health and response of these cardiac myocytes. DETA, fibronectin and Entactin-Collagen-Laminin (ECL) matrix were the surfaces studied. Preliminary results have shown a healthier monolayer and higher beat frequency with serum free conditions. In the comparative study between different surfaces, best results have been obtained on DETA for beat frequency.

BI-TuP20 In-vitro Electrophysiological Comparison of Embryonic Hippocampal Neurons Grown in 2D and 3D Environments, ***T. Xu***, *P. Molnar, C.A. Gregory, M. Das, J.J. Hickman, T. Boland,* Clemson University

To compare electrophysiological differences between neurons cultured in 2D and 3D environments, neurons dissociated form embryonic rat brain were seeded onto type-I collage thin coating, collagen gel surface, and were entrapped randomly into collagen gel, which were set up for imitating a 2D culture environment, a defined 3D environment and a random 3D environment for neuron culture respectively. Double-immunostaining for MAP-2, a neuronal cell body and dendritic marker, and anti-neurofilament antibody, an axonal marker, was used to identify neuron morphology. Hippocampal neuron polarities and outgrowth of neurites were evaluated by confocal microscope images. The gels were optimized for neuronal 3D cultures by varying concentrations, porosities, and glial cell densities. Surface properties of the gels will be characterized by AFM. Whole-cell patch clamp experiments were carried out to investigate electrophysiology of hippocampal neurons cultured in different conditions. Improvements of patch clamp technique for neuronal 3D culture over traditional 2D culture were applied. Membrane and synaptic properties of neurons in response to their different culture conditions were recorded and compared. Our preliminary results show that hippocampal neurons cultured in 2D and 3D environments exhibited similar passive membrane properties and sodium and potassium currents. Repetitive firings of action potential were found in neurons cultured in 3D environment over 14 days. Results will be presented on functional synapse formation for neurons cultured in 3D environment as measured by the combination of the patch clamp technique and the sharp microelectrode technique. The results of this study indicate that embryonic hippocampal neurons retain a clearly neuronal electrophysiological phenotype in a engineered in-vitro 3D culture condition, which is holding potential in applications ranging from neural tissue engineering to providing active neuronal networks for neuro-computing.

BI-TuP21 Detection of E. Coli O157:H7 with Surface Plasmon Resonance Biosensor in Complex Matrices, ***A. Taylor***, *Q. Yu, S. Jiang,* University of Washington

There is an urgent need for fast, sensitive, and reliable methods for detecting biological warfare agents and food contaminants. Large analytes (i.e. Escherichia coli, Salmonella enteritidis, or Listeria monocytogenes) are difficult to detect and quantify at low concentrations without time-consuming amplifications (i.e. culturing and PCR). E. coli O157:H7, an important food contaminant, was detected with a surface plasmon resonance (SPR) biosensor. However, with amplification, the detection limit was 5x10@super 7@ cfu/ml. In this work, we detect E. coli in both buffer and complex matrices using a home-built SPR. Antibody specific to the O antigen protein expressed on the membrane of the E. coli cell was immobilized on sensing surface via self-assembled monolayers. Atomic force microscopy (AFM) is combined with SPR to optimize surface chemistries and antibody immobilization at the molecular level. Transport of large charged bacteria to the antibody functionalized surface is one important factor limiting the ability to detect low concentrations. Thus, we study the effects of flow rate and pattern on detection. The biosensor was proven to differentiate between E. coli strains O157:H7 and K12 based on the antibody chosen. Furthermore, immunomagnetic separation methods using antibody functionalized magnetic particles were used to separate analytes from complex matrices (i.e. ground beef). The objectives of this work are to improve low detection limit and to minimize non-specific binding for SPR detection of larger-sized analytes in complex matrices.

BI-TuP22 A Comparative Study of Bone Cell Attachment and Spreading on Various Metal Surfaces by Cryo-SEM and QCM-D, ***M. Foss***, *J. Justesen, M. Duch, A.-L. Stranne, J. Chevallier, C. Modin, F.S. Pedersen, F. Besenbacher,* University of Aarhus, Denmark

The detailed understanding of the attachment of bone-forming cells on surfaces is crucial for the development of new generations of orthopaedic implant materials. The goal of these studies is to establish methods for a more quantitative measurement of biocompatibility. Here, traditional methods of cell counting and cell area measurements are correlated with data obtained by the Quartz Crystal Microbalance technique (QCM-D, Q-Sense AB) of cell attachment and spreading. The spreading and attachment of the murine preosteoblastic MC3T3-E1 cells were quantified by cryo-SEM. Cells were seeded and allowed to attach for various periods of time, fixed and snap-frozen in liquid nitrogen. The number of attached cells and the mean area were determined using a standard image analysis program. After attachment the shape of the cells changes from round to an initial maximum spread at the surface. The time-scale for maximum spread at serum-free conditions has been determined to 40 - 50 min, which is in good agreement with QCM-D data where the maximum shifts in both frequency and dissipation are reached at similar time points. The examinations have been applied to several relevant metal surfaces including tantalum and chromium. At cell concentrations ranging from 50,000 cells/ml to 300,000 cells/ml, a variation in the degree of cell spreading is observed between these two metals implying differences in the cell attachment at the chemically different surfaces. However, the viscoelastic properties of the cells are independent of the substrate material. The results point to the establishment of a fast and accurate general method for screening biomaterials with QCM-D. The methods will furthermore be applied to surfaces functionalized by prototype proteins including BSA and fibronectin.

BI-TuP23 Control of Osteopontin Behavior on Surfaces for Cell Adhesion, ***L. Liu***, *S. Chen, B.D. Ratner, S. Jiang,* University of Washington

Osteopontin (OPN) is an important extracellular matrix protein shown to function in wound healing, inflammation and foreign body reaction and has been identified as a potential target for engineered biomaterials. It contains RGD moiety that has been shown to mediate cell adhesion through interaction with integrins. In preferred orientation and conformation, the RGD tripeptide of OPN will be presented to the cells to the greatest extent. However, control of OPN orientation/conformation is seldom investigated so far. In this work, we investigate OPN adsorption and cell adhesion on self-assembled monolayers (SAMs) of alkanethiols terminated with different functional groups to tailor surface properties. Four different alkanethiols terminated with -CH3, -OH, -NH2 and -COOH were used to form surfaces representing hydrophobic, hydrophilic, positive and negative surfaces. Atomic force microscopy (AFM) is used to characterize the adsorption of OPN on various SAM surfaces. Our AFM results show that the amount of adsorbed OPN on -COOH surface is slightly less than that on -NH2 surface. In vitro cell adhesion assay of bovine aortic endothelial cells (BAEC) was performed to test OPN function on various SAMs. Our results show that on -NH2 surface BAEC adhesion is the most and cell appears most spread. Both cell counts and average cell spreading area on -COOH and -CH3 surfaces are much less than those on -NH2 surface. The -OH surface is resistant to both OPN adsorption and cell adhesion. By comparing results from AFM and cell adhesion experiments, it is suggested that the orientation/conformation of OPN on -NH2 positively charged surface is more favorable for cell interaction than on -COOH negatively charged surface. This is consistent with our molecular simulation prediction. Our studies clearly show that surface properties will alter OPN behavior on surfaces, thus cell interactions with OPN. In addition, we use atomic force microscopy (AFM) to image the binding of OPN onto type I collagen monomer. Interactions among extracellular matrix (ECM) proteins are important in many aspects such as orientating ligand proteins and correctly delivering signals into a cell. Recent experiments show that osteopontin (OPN)-incubated collagen I chemically immobilized on poly(HEMA) promotes cell adhesion in vitro. It is speculated that bound OPN on a collagen matrix may have better orientation/ conformation and thus influence its cell-binding ability. Although it has been reported that OPN is able to bind with type I collagen, direct visualization of OPN-collagen binding complexes has not been reported and OPN binding sites on collagen I are still unknown. In our work, AFM is used to image the binding of OPN onto individual triple-helical collagen I monomer on freshly cleaved mica for the first time. Analysis of AFM results clearly shows binding patterns of OPN to collagen I. This work provides a direct means to identify binding among ECM proteins and a better understanding of ECM proteins on cell adhesion.

BI-TuP24 Tissue Formation of Endothelial Cells on a Microporous Film of Biodegradable Polymer, ***T. Nishikawa***, *T.A. Ohzono, J. Hayashi, M. Hara, M.A. Shimomura,* The Institute of Physical and Chemical Research, Japan

Micropatterned surface is considered to be a promising biointerface that can control both surface chemistry and surface morphology of cell culture substrates. The biointerface to be issued in this report is a microporous film of biodegradable polymers. Honeycomb films are microporous films of polymers which are formed by applying moist air to a spread polymer

solution. We report the tissue formation of endothelial cells (ECs) on self-supporting honeycomb films. The tissue formation was studied in regard to cell-matrix adhesion, proliferation, and movement. Honeycomb films were prepared from mixtures of biodegradable polymers (poly(L-lactic acid) (PLLA) and poly(@epsilon@-caprolactone) (PCL)) and amphiphilic polymers). Adhesion behavior of ECs was characterized by formation of stress fiber of actin and localization of focal adhesion proteins at the interface between cells and culture substrate. ECs did not form focal adhesions on self-supporting microporous films. The modulated cell adhesion on the microporous films influenced cell-division cycle of ECs. Doubling time represents an average period of cell-division cycle. The doubling time of ECs estimated from the proliferation curves was 20 hrs on flat cast film of PCL and 27 hrs on microporous films of PCL. Micropores can be considered to be pathways connecting two sides of a self-supporting honeycomb film of PLLA. ECs were seeded onto a top side of a honeycomb film having an average poresize of 5 μm and an average thickness of 3 μm. At the day 11 of culturing, the cell culture was observed by confocal microscopy after staining filamentous actin of ECs and a honeycomb film with fluorescent probes. Monolayer of ECs was confirmed at each side of the honeycomb film. This suggests that ECs attached onto the top side pass through the micropores, appear on the bottom side of a honeycomb film, grow, proliferate, and finally cover the both sides of the honeycomb film.

Biomaterial Interfaces
Room 307 - Session BI+SS-WeM

Cell Interactions with Patterned Surfaces
Moderator: M. Textor, ETH Zurich, Switzerland

8:20am **BI+SS-WeM1 Patterned Surfaces using Masking during Plasma Deposition or Pulsed Laser Ablation**, *H. Thissen,* CSIRO Molecular Science, Australia; *J.P. Hayes,* Industrial Research Institute Swinburne, Australia; *P.G. Hartley, G. Johnson,* CSIRO Molecular Science, Australia; *E.C. Harvey,* Industrial Research Institute Swinburne, Australia; ***H.J. Griesser,*** University of South Australia, Australia

The patterning of biomaterial surfaces has attracted much recent interest for various fundamental and applied purposes, such as the control of the location and shape of attached anchorage-dependent cells. Patterned surfaces are also of interest for bio-diagnostic arrays, cell culturing and separation, some tissue engineering products, and some biomedical implants. We have used two different approaches for the fabrication of patterned surface chemistries. One approach involves the use of masks during the deposition of thin plasma polymer coatings. The other approach is based on the deposition of multilayer coating structures followed by laser ablation through a mask; the top layer is a non-adhesive coating such as PEG and the laser beam exposes adhesive regions "underneath" by ablating the PEG layer in spatially controlled areas. Cell-adhesive proteins can then adsorb only onto the exposed areas capable of adsorbing proteins. The second approach is very attractive because of its speed and ease of fabrication; ablation of the thin PEG layer using a pulsed 248 nm excimer laser is fast with nanometre thickness control by controlling the number of laser pulses. The patterned surface chemistries and their protein adsorption characteristics were analyzed by several surface analytical techniques and by antibody assay. Cell culture using bovine corneal epithelial cells confirmed that cell attachment is controlled by these surface chemistry patterns. Our work has so far focused on fluoropolymer and Si wafer substrates and the use of plasma polymer interlayers for the covalent anchoring of a cloud point grafted PEG top layer; the use of a plasma polymer interlayer has the advantage of being readily transferable to a variety of substrates both ceramic and polymeric. However, the use of laser patterning is not restricted to those coating structures and can be applied to burn adhesive "holes" into other non-adhesive coatings equally well.

8:40am **BI+SS-WeM2 Patterning Surfaces with "Nonfouling" Oligoethylene Glycol "Bottle Brushes" by Soft Lithography and Surface-Initiated Atom Transfer Radical Polymerization**, ***H. Ma,*** *A. Chilkoti,* Duke University

A "grafting from" strategy is described for creating patterned biologically-nonfouling polymer coatings. Initiators presenting a bromoisobutyrate moiety and a thiol group at two ends of the molecule were synthesized and patterned on gold by soft lithography. The patterned SAM was used as a substrate for surface-initiated atom transfer radical polymerization (SI-ATRP) of oligoethylene glycol methyl methacrylate (OEGMA). The SI-ATRP was carried out in an oxygen-free environment with CuBr/Bipy as catalysts in a water /methanol mixture. Ellipsometry showed that the thickness of the poly(OEGMA) "bottle brush" could be easily manipulated from 0 to 50 nm by control of the polymerization conditions. The patterns were characterized by imaging ToF-SIMS, imaging XPS, and AFM. This "bottle brushes" are exceptionally protein-resistant. Surface plasmon resonance (SPR) spectroscopy showed no adsorption of fibronectin (1 mg/ml), 10% or 100 % fetal bovine serum (FBS) onto those surfaces. NIH 3T3 fibroblasts were confined to regions demarcated by the patterned poly(OEGMA) brushes. The cellular patterns were maintained for over 30 days, which is significantly longer than is possible with EG-terminated alkanethiol SAMs. This "grafting from" strategy is not limited to gold-coated surfaces as demonstrated by SI-ATRP on glass and silicon, and overcomes the intrinsic limitation of low surface density of PEG chains by physisorption or the "grafting to" approach. The poly(OEGMA) grafts synthesized in situ by SI-ATRP recapitulate in a polymer brush some of the key features of oligoethylene glycol-terminated SAMs, namely the high surface density of oligoethylene glycol in a thicker and more robust coating. These patterned "nonfouling" surfaces have utility in the design of experimentally useful model system to investigate the response of cells to chemical and topographical cues, in addition to a wide range of applications in bioanalytical devices.

9:00am **BI+SS-WeM3 Molecular Assembly Patterning by Lift-off (MAPL): A Novel Approach to Produce Biologically Designed Micropatterns for Biosensor Applications and Cell-Surface Interaction Studies**, ***D. Falconnet,*** Swiss Federal Institute of Technology (ETH) Zurich; *F. Assi,* Swiss Federal Institute of Technology (ETH) Zurich, Switzerland; *A. Koenig,* Swiss Federal Institute of Technology (ETH) Zurich; *M. Textor,* Swiss Federal Institute of Technology (ETH) Zurich, Switzerland

A new chemical micropatterning technique is presented for cell-surface interaction studies. The MAPL technique allows creating patterns of bioactive molecules (such as biotin, peptides, oligonucleotides) at a controlled surface density and embedded in a background resistant to the adsorption of proteins. A simple photoresist lift-off process is exploited in conjunction with the spontaneous assembly of polycationic poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) onto negatively charged metal oxide surfaces. A positive photoresist on a metal-oxide-coated substrate (e.g. niobium oxide coated on glass) is developed resulting in a micropattern of resist and bare metal oxide areas. Bio-functionalized (e.g., biotin or cell-adhesive peptide) PLL-g-PEG is immobilized at the bare metal oxide regions by spontaneous assembly from aqueous solutions of the polymer. The photoresist is lifted off in an organic solvent without affecting the integrity of the adsorbed functionalized PLL-g-PEG monolayer. Subsequently, the background is backfilled with protein- and cell-resistant PLL-g-PEG. The resulting pattern of bio-interactive and non-adhesive areas shows an excellent contrast on the protein level, demonstrated by fluorescence microscopy using labeled streptavidin to specifically decorate the PLL-g-PEG/PEG-biotin patches. Cell attachment to such micropatterns consisting of PLL-g-PEG/PEG-RGD-peptide was tested using human foreskin fibroblasts. This lift-off-based biochemical patterning is a 'soft', robust, simple and reproducible technique that does not require specialized clean room and heavy etching facilities. It is an interesting alternative to microcontact printing because it circumvents many disadvantages of the printing technique. The high signal-to-noise ratio and the feasibility of tailoring the bioligand (capture molecule) density in the interactive patches make MAPL a promising technique also for biosensor microarray applications.

9:20am **BI+SS-WeM4 Micrometer-scale Fibronectin Patterning for Control of Focal Adhesion Dynamics in Fibroblasts**, ***D.S. Rhoads,*** *R.N. Orth, M. Wu, B.A. Baird, J.L. Guan,* Cornell University

We have developed a new method for analyzing the processes of fibroblast adhesion and spreading using micro- and nanometer-scale fibronectin patterns. Fibronectin is an extracellular matrix protein that provides mechanical stability for cells and tissues, by being a ligand for integrin cell surface receptors which anchor the actin cytoskeleton to the plasma membrane. These anchor points are referred to as focal adhesions, and are composed of numerous scaffolding and signaling proteins in addition to forming focus points of the actin cytoskeleton. Here, we patterned fibronectin using a technique previously shown to produce feature sizes as small as 700nm. The fibronectin features are used to observe small focal adhesions and the morphological effects of minimal activation by fibronectin per cell area. For fabrication of patterned surfaces, polymer-coated silicon wafers were patterned using photolithography and reactive ion etching. Fibronectin was then deposited onto the wafer samples prior to polymer removal and cell application. The resulting patterns contained features ranging from 76 µm to > 1 µm, and were used in cell adhesion and spreading experiments. Cells adhering to the pattern were fixed, permeablized and analyzed by immunofluorescence, using antibodies to fibronectin, f-actin, paxillin, and focal adhesion kinase. Fluorescence microscopy was complemented with scanning electron microscopy to image focal adhesions, stress fibers, lamellipodia and filopodia. From this analysis, we propose that this method for analyzing cellular responses to subcellular cues from their surroundings is a model system for spatially isolating and studying focal adhesions.

9:40am **BI+SS-WeM5 Microengineering Surfaces to Interface with Mammalian Cells**, ***C.S. Chen,*** Johns Hopkins University **INVITED**

The interactions between cells and their surroundings provide the basis for the coordinated functions of tissues. To understand and control these interactions, we have developed several microfabrication-based approaches to provide model environments for cells. We will describe these approaches, and how they are beginning to elucidate how cells probe and make sense of their environment through biochemical and mechanical means. Integrating microfabricated devices and cells will pave the way for a new era in biomedical research and medicine.

10:20am **BI+SS-WeM7 Analyzing Lymphocyte Adhesion, Membrane Receptors and Cytoskeletal Rearrangement on Micron Scale Mitogen Patterns**, ***R.N. Orth***, *M.J.B. Flaminio, J. Kameoka, T.G. Clark, H.G. Craighead,* Cornell University

In this study, we investigated an in vitro immune reaction on a planar surface between T cells, B cells, and micron scale patterned mitogens as a model system for analyzing cell surface ligand responses. To form functionalized biomaterial microdomains, a polymer-coated substrate was patterned using photolithography and reactive ion etching. The samples were incubated in antibody and mitogen solutions prior to polymer removal and cell application. Uniform mitogen patterns ranging from 76 µm to <1 µm were created to target cell surface receptors, upregulate intracellular signaling cascades and cell activity, and stimulate proliferation. Several methods were used to analyze the patterned mitogens' effects on the lymphocytes. Carboxy-fluorescein diacetate, succinimidyl ester (CFSE)-stained lymphocytes harvested from the substrate demonstrated a proliferative response when assayed by flow cytometry. Mouse monoclonal antibodies against equine membrane cell receptors (anti-major histocompatibility (MHC) class II, anti-CD4, anti-CD3, and anti-leukocyte function associated-antigen (LFA)-1) provided a view of stimulated cells' surface receptor distribution. Secondary anti-mouse antibodies with a conjugated 1.5 nm gold sphere were bound to the primary antibodies. The samples were incubated in a silver solution to form 10-100 nm spheres as the silver nucleated off the gold particles. Scanning electron microscopy (SEM) imaging provided high resolution images of the cell surface ligands' spatial distribution as marked by the silver spheres. This patterning technique provided a precise and reproducible means to structure biomaterial surfaces at subcellular resolutions.

10:40am **BI+SS-WeM8 Directed Motoneuron Growth on Self-Assembled Monolayer (SAM) Patterned Surfaces**, ***M.G. Poeta***, *M. Das, C.A. Gregory, P. Molnar, D.C. Henry, L.M. Riedel, J.J. Hickman,* Clemson University

We are investigating the directed growth of embryonic rat motoneurons on glass substrates and are determining if they exhibit proper morphological and electrophysiological characteristics in this defined environment. This is the first step in recreating the reflex arc, one of the fundamental controls circuits in the body, with biological components on a MEMS chip. Recreating this system in vitro could have significant implications for improving treatment for people with spinal cord injuries, which affect 10,000 people every year in the United States. Throughout the last century, many methods have been developed to direct the growth of different cell types. These include fibroblasts, glial cells and hippocampal neurons grown on spider webs adhered to coverslips, grooves scratched in polystyrene and palladium deposited on petri dishes. In order to direct the growth of the motoneurons, we are using two recently developed patterning techniques, microcontact printing and laser ablation. Microcontact printing is a patterning method where a polydimethyl siloxane (PDMS) stamp is cast from a mold. It is inked in our case with a hydrophilic silane, diethyltriamine trimethoxysilane (DETA), and brought into contact with a substrate. The substrate is then backfilled with a hydrophobic silane, tridecafluoro-1,1,2,2-tetrahydroctyl-1-trichlorosilane (13F). Laser ablation is a patterning method where, again in our system, a substrate is coated with DETA and exposed with an excimer laser fitted with a beam homogenizer through a photomask. The exposed regions are then backfilled with 13F. Embryonic rat motoneurons are plated on these patterned substrates. XPS and contact angle are used to verify the surface modification procedures. We have found that the motoneurons orient themselves along the hydrophilic patterns. We will report on the characterization of these patterns using patch-clamp electrophysiology to measure the electrophysiological characteristics of the cells.

Biomaterial Interfaces
Room 317 - Session BI-WeM

Bionanoscale Analysis: Theory to Experiment

Moderator: R.A. Latour, Clemson University

8:20am **BI-WeM1 Calculation of Free Energy of Peptide-Surface Adsorption Using Molecular Dynamics Simulations**, ***V. Raut***, *S.J. Stuart, R.A. Latour,* Clemson University

Proteins, which are bioactive molecules, adsorb on implants placed in the body and directly influence biocompatibility. Molecular dynamics (MD) modeling provides one of the most direct methods of analyzing individual molecular-level interactions and can be used to simulate protein adsorption behavior using empirical force fields. In order to correctly simulate protein adsorption behavior, a force field must correctly represent the thermodynamic driving forces governing peptide residue-surface interactions (i.e., adsorption enthalpy, entropy & free energy). However, since existing force fields were developed without consideration of protein adsorption, they may not accurately represent this type of molecular behavior. Therefore the objective of our research is to develop computational chemistry methods to calculate thermodynamic parameters of peptide-surface adsorption and compare them with experimental results for the assessment of force field accuracy. Various MD simulations demonstrating individual residue-surface reactions are being studied. These models represent the behavior of small peptides over functionalized SAM surfaces in a water box with periodic boundary conditions. Statistical mechanics methods are being developed based on positionional probability distributions obtained from MD simulations to enable us to calculate the change in enthalpy, entropy & free energy as a function of distance between the peptide & surface. Comparison of these results with experimental results will enable us to determine the accuracy of available force fields. If necessary, the developed methods will then also serve as a basis for the development of a new force field that is specifically parameterized to accurately simulate protein adsorption behavior.

8:40am **BI-WeM2 Molecular Simulation Studies of Protein and DNA Interactions with Surfaces**, *J. Zheng, J. Zhou, J.P. Sullivan, L. Zhang,* ***S. Jiang***, University of Washington

Molecular-level understanding of protein behavior on surfaces will facilitate the development of biomaterials with superior biocompatibility and biosensors with high sensitivity and specificity. In this work, we report various molecular simulation studies of non-fouling mechanism, protein orientation/conformation on surfaces, molecular recognition, and DNA chips. First of all, molecular dynamics (MD) simulations are performed to study lysozyme interactions with SAMs presenting oligo (ethylene glycol) (OEG) groups in the presence of explicit water molecules and ions. The behavior of water at protein/SAM interfaces is characterized by self-diffusion coefficient, order parameter, hydrogen bonding, and radial distribution. The effects of surface (charge, hydrophobicity, and defect), solvent, pH, and ion strength will be taken into account. Results from this work will shed light on non-fouling mechanism at the atomic-scale level and guide the design of better biocompatible materials and biosensors. Second, Monte Carlo simulations are performed to study IgG orientation on positively charged NH2 and negatively charged COOH terminated self-assembled monolayers (SAMs) on Au(111). Simulations are confirmed by experimental results from surface plasmon resonance (SPR) biosensor and time-of-flight secondary ion mass spectrometry (ToF-SIMS). Third, MD simulations are performed to study molecular conformation of cytochrome c on charged SAMs in the presence of explicit water molecules and ions. The ability to predict protein orientation and conformation will enable one to control and manipulate protein behavior on surfaces, important for biomaterials and biosensors. Fourth, hybrid molecular simulations are performed to study the unbinding pathway of biotin/avidin interactions at the atomic force microscopy time scale. Finally, MD simulations are performed to study the molecular packing of thiolated ssDNA and dsDNA on Au(111) and then mixed DNA and OEG SAMs.

9:00am **BI-WeM3 Macromolecular Dynamics: Insights from Simulation**, ***B.R. Brooks***, National Institutes of Health **INVITED**

Examples of recent macromolecular simulations will be presented which explore the relationship between modeled systems and real systems probed by experiment. In particular, data from neutron scattering, NMR, and crystallography can be compared and contrasted with corresponding results from molecular dynamics simulation. Also presented will be examples where simulation can provide insights that are difficult to obtain with experiment. There will be some discussion of recently developed methods that enhance our ability to accurately model interfacial systems and interactions between macromolecules. Also, protein conformational change and enzyme catalysis can be examined with a variety of methods. We present several methods, such as the Replica/Path method and extensions of the Nudged Elastic Band method, for examining such events and their application to interesting biological systems.

9:40am **BI-WeM5 Molecular Modeling of Adsorption-Induced Exposure of Integrin Binding Sites in Fibrinogen**, ***M.A. Agashe***, *S.J. Stuart,* Clemson University*; L. Tang,* The University of Texas at Arlington*; R.A. Latour,* Clemson University

Implants invoke inflammatory responses from the body even if they are chemically inert and non-toxic. It has been shown that a crucial precedent event in the inflammatory process is the spontaneous adsorption of

fibrinogen on implant surfaces, which is typically followed by the presence of phagocytic cells. It has been found that interactions between the phagocyte integrin Mac-1 and one short sequence within the fibrinogen D domain (@gamma@190 to 202) partially explain phagocyte accumulation at implant surfaces. However, it is still unknown what makes adsorbed fibrinogen proinflammatory when soluble fibrinogen is not. One premise is that adsorption exposes the normally occult P1(@gamma@190 to 202) and P2 (@gamma@377 to 395) epitopes that reside in the D domains of fibrinogen; these epitopes are also involved in thrombin-mediated conversion of fibrinogen to fibrin. The objective of our research is to use molecular modeling to investigate how surface chemistry influences the adsorption behavior of the D fragment of fibrinogen with a particular focus on characterizing adsorption-induced conformational changes in the P1 and P2 region of this fibrinogen fragment that may lead to epitope exposure for integrin binding. Modeling is being conducted using Insight II software (Accelrys) with the CHARMM force field. The adsorption of the @gamma@ chain of fibrinogen is being simulated on 4 types of SAM surfaces (hydrophobic, hydrophilic, + - charged). An implicit solvent model (generalized Born) is being used to represent the solvent and solvent-mediated interactions during the molecular dynamics simulations. The study of these changes in conformation will help us to understand the likely molecular mechanisms that are responsible for the exposure of the P1 and P2 domains, and how this may be able to be controlled by surface chemistry. This understanding may help in the design of biomaterial surfaces with improved biocompatibility.

10:00am **BI-WeM6 Scaled Interfacial Activity of Proteins at the Liquid-Vapor Interface***, **A. Krishnan**, J. Sturgeon, C.A. Siedlecki, E.A. Vogler,* The Pennsylvania State University

A principal conclusion drawn from observations of time- and concentration-dependent liquid-vapor (LV) interfacial tension @gamma@@sub lv@ of a diverse selection of proteins ranging from albumin to ubiquitin is that concentration scaling substantially alters perception of protein interfacial activity, as measured by the amount adsorbed to the hydrophobic LV surface. Proteins appear more similar than dissimilar on a weight/volume basis whereas molarity scaling reveals a "Traube-rule" ordering by molecular weight, suggesting that adsorption is substantially driven by solution concentration rather than diversity in protein amphilicity. Scaling as a ratio-to-physiological-concentration demonstrates that certain proteins exhibit the full possible range of interfacial activity at-and-well-below physiological concentration whereas others are only weakly surface active within this range, requiring substantially higher solution concentration to achieve maximum adsorption to the LV interface. Important among this latter category of proteins are the blood factors XII and XIIa, assumed by the classical biochemical mechanism of plasma coagulation to be highly surface active, even in the presence of overwhelming concentrations of other blood constituents such as albumin and immunoglobulin that are shown by this work to be among the class of highly-surface-active proteins, at physiologic concentration. A comparison of pendant-drop and Wilhelmy-balance tensiometry as tools for assessing protein interfacial activity shows that measurement conditions employed in the typical Wilhelmy plate approach fails to achieve the steady-state adsorption state that is accessible to pendant-drop tensiometry. A comparison of bovine and human proteins reveals substantial differences in adsorption to the LV interface, apparently arising from as-yet unresolved speciation effects.

10:20am **BI-WeM7 Nanodevices Integrating Biomolecular Motors: Design Strategies and Applications***, **H. Hess**, J. Clemmens,* University of Washington*; C. Matzke, G.D. Bachand, B.C. Bunker,* Sandia National Laboratories*; V. Vogel,* University of Washington

Biomolecular motors are at present the engines of choice for nanodevices.@footnote 1,2@ Their small size, high efficiency, and functional integration allow the construction of hybrid devices, which demonstrate the promise of engineering at the nanoscale. We will discuss the tools employed in designing these devices, which include surface patterning, microfabrication, and genetic engineering. Our recent results@footnote 3@ show that these tools have to be employed in concert, in order to achieve outstanding results. For example, controlled placement of motor proteins on a surface requires non-fouling regions of high quality, as well as fine-tuning of the adsorption properties of the motors by genetic engineering. The design process in general requires an in-depth understanding of the motor properties as well as the properties of the filaments the motor proteins bind to (e.g. microtubules). New measurements aim at determining these properties. We will also present an overview of the applications studied by us, ranging from molecular shuttles@footnote 4,5@ to surface imaging @footnote 6@ and force measurements.@footnote 7@ @FootnoteText@@footnote 1@ H. Hess and V. Vogel, Reviews in Molecular Biotechnology, 82, 67-85 (2001)@footnote 2@ H. Hess, G. Bachand, and V. Vogel in: Encyclopedia of Nanoscience and Nanotechnology. Edited by James A. Schwarz, Cristian Contescu, and Karol Putyera (Marcel Dekker, New York, in print)@footnote 3@ J. Clemmens, H. Hess, J. Howard, V. Vogel, Langmuir, 19, 1738-1744 (2003)@footnote 4@ H. Hess, J. Clemmens, D. Qin, J. Howard, and V. Vogel, Nano Letters, 1 (5), 235-239 (2001)@footnote 5@ H. Hess, J. Clemmens, C. M. Matzke, G. D. Bachand, B. C. Bunker, and V. Vogel, Appl. Phys. A, A 75, 309-313 (2002)@footnote 6@ H. Hess, J. Clemmens, J. Howard, and V. Vogel, Nano Letters, 2 (2), 113-116 (2002)@footnote 7@ H. Hess, J. Howard, and V. Vogel, Nano Letters, 2(10), 1113-5 (2002).

10:40am **BI-WeM8 Nanoparticle Transport Using Microtubules and Motor Proteins***, **B.C. Bunker**, G.D. Bachand, A.K. Boal, S.B. Rivera, T.J. Headley, J.M. Gaudioso, J.M. Bauer, R.P. Manginell,* Sandia National Laboratories*; H. Hess, V. Vogel,* University of Washington

Active transport systems consisting of motor proteins and microtubules can potentially provide a dynamic mechanism for assembling and reconfiguring materials at nanometer length scales. We are interested in using motor protein-microtubule systems to manipulate gold nanoparticles and quantum dots to create programmable or responsive conductive or optical arrays within microfluidic systems. The primary active transport strategy we have investigated involves the use of patterns of tethered motor proteins to transport short functionalized microtubules attached to nanoparticles. This talk will focus on two central issues associated with developing a viable transport system: 1) the development of surface functionalization schemes that optimize the guiding of microtubule shuttles through lithographically-defined networks, and 2) the development of functionalized microtubule configurations that allow nanoparticles to be carried without affecting critical motor protein-microtubule interactions. For guiding, we have obtained the best results using lithographic patterns containing both gold and silica surfaces. The gold surfaces are coated with self-assembled monolayers (oligoethylene glycol and amine terminations are most effective) that are antifouling with regard to proteins, confining the adsorption of motor proteins and their support structures onto exposed silica at the channel bottoms. In terms of microtubule functionalization, we have demonstrated that both gold nanoparticles and CdSe quantum dots can be attached to microtubules using standard biotin-streptavadin linkages. The structures of the nanoparticle-microtubule constructs have been characterized using both transmission electron and atomic force microscopies. Flourescence microscopy results show that the number and spatial distributions of particles must be controlled to achieve active transport. Several successful strategies for controlling such distributions will be described.

Biomaterial Interfaces
Room Hall A-C - Session BI-WeP
Poster Session

BI-WeP1 Locally Addressable Electrochemical Patterning Technology (LAEPT) using Poly(L-lysine)-g-Poly(ethylene glycol), PLL-g-PEG, ***C.S Tang***, Swiss Federal Laboratories for Materials Testing and Research, Switzerland

Protein-resistant polyelectrolyte, poly(L-lysine)-g-poly(ethylene glycol) PLL-g-PEG adsorbs spontaneously onto a substrate with surface contrast constituting of conductive titanium and non-conductive silicon-oxide. An applied potential between -0.4 and +1.7V removes the PLL-g-PEG from titanium but simultaneously, there was insignificant polyelectrolyte loss on the silicon-oxide. X-ray photoelectron spectroscopy confirmed the reduction of PLL-g-PEG on the titanium surface and it also indicated that approximately similar amount of PLL-g-PEG remained on the titanium oxide when low corresponding positive and negative voltages of up to 400mV were applied. At 1.7V, time-of-flight secondary ions mass spectroscopy and fluorescence microscopy distinctly demonstrated the intensity contrast between the retention of PLL-g-PEG on the silicon-oxide and PLL-PEG removal from titanium. It is believed that the native oxide layer of titanium undergoes morphological changes with ascending potential and this affects the adhesion stability of PLL-g-PEG on the titanium oxide surface. Electrochemical impedance spectroscopy monitored the voltage-induced changes in the oxide layer whose measured impedance and resistance were found to decrease dramatically with increasing voltage. Further investigations hinted that diffusional-controlled processes within the oxide caused complex morphological changes, eventuating in an unstable adhesion platform for weak PLL-g-PEG electrostatic binding. The difference in the response of an applied potential on the titanium/silicon region under electrochemical conditions permits the exploitation and regeneration of various immobilization techniques on titanium while maintaining a protein resistant background on the non-conductive region. This reliable method offers prospects in selective electrochemical patterning for the biomedical as well as semiconductor industries. It will be termed here as locally addressable electrochemical patterning technology, LAEPT.

BI-WeP2 Simple Fabrication of Polymer Thin Films with Lithographic Bas-relief Micro-pattern and Self-organized Micro-porous Structure, ***T.A. Ohzono***, *T. Nishikawa, M.A. Shimomura,* RIKEN, Japan

The cost of making micro scale components through conventional lithographic techniques increases depending on the degree of design complexity. Whereas, non-lithographic approaches have also been investigated extensively to reduce or replace the complicated process involved in those lithographic techniques. Therefore, it seems necessary to combine the good aspects of the self-organization process and of the conventional lithography toward the optimum productivity for fabrication of micro scale textures for some practical applications . Adopting such approach, here we show a very simple method for fabrication of a patterned polymer thin film with a hierarchical structure. The structure consists of a bas-relief pattern at tens of microns and the ordered array of pores with diameters of 4-5 μm. The former pattern is originally fabricated through conventional photolithography. The latter emerges from self-organized process, where micrometer-size water droplets condensed on the surface of evaporating solutions are spontaneously arranged. The film is self-supporting. It is possible to control by the polymer concentration whether the film is bottomless, partially bottomless, or not. The bio-compatible polymer of the lactic-acid can be used as the material. The film with the novel structure will enable us to do patterning of functional particles, of cells, and of bio-sensing elements toward new bio-coupled devices.

BI-WeP3 Adsorption Kinetics of Alkanethiol Self-Assembly on Hydrogenated Ge(111), ***M.R. Kosuri***, *R. Cone, Q. Li, S.M. Han,* University of New Mexico*; C.B. Bunker, T.M. Mayer,* Sandia National Laboratories

We have investigated in situ and in real-time the liquid-phase self-assembly of 1-alkanethiols on hydrogenated Ge(111), using attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIRS). The water contact angle measurements on thiolated Ge demonstrate that the final packing density is a function of both alkanethiol concentration in 2-propanol and the chain length of thiolate molecules. The absolute saturation coverage of 1-hexadecanethiol is approximately 4.2x10@sup 14@ cm@sup -2@ based on the IR absorbance of C-H stretching vibrational modes near 2900 cm@sup -1@. We also report the adsorption rate constant of 0.1 M 1-hexadecanethiol on hydrogenated Ge(111) at room temperature. The rate constant is 2.1 ± 0.6 cm@sup 3@/mol-sec, based on a Langmuir isotherm.

BI-WeP4 Deposition of Lipid DPPC Monolayer on SiO@sub 2@ Surface using OTS Self-assembled Monolayer Islands as Anchor Molecules, ***M. Takizawa***, *Y.H. Kim,* The Graduate University for Advanced Studies, Japan*; T. Urisu,* The Graduate University for Advanced Studies and Institute for Molecular Science, Japan

Bilayer lipid membranes (BLMs) supported on the gold surface are active research target from the viewpoint of application to the biosensors. It is reported that the stability of the membrane can be extended significantly by using the "anchor molecules", i.e. the synthesized thiolipid which is chemically anchored to the gold surface. In this work, we have examined for the first time the deposition of DPPC (dipalmitoyl phoshatidylcholine) monolayer on SiO@sub 2@ surface using OTS (n-octadecyltrichlorosilane) self-assembled monolayer (SAM) islands as anchor molecules. OTS SAMs have been deposited by dipping the Si (111) substrates with thermally oxidized SiO@sub 2@ surface layer into ~10 % toluene solution (containing small amount of water) at a room temperature. After deposition of OTS SAM island, the DPPC monolayer was transferred to the substrates by Langmuir-Brodgett method at the surface pressure of 35 mN/m. The height of the OTS SAM island measured by AFM was ~2 nm, which is consistent with a previous report. The surface morphology measurements by AFM after the DPPC transfer shows that the flat DPPC monolayer is deposited almost completely filling the (hydrophilic) SiO@sub 2@ surface area-selectively. On the (hydrophobic) OTS SAM island surface, on the other hand, DPPC monolayer deposition was not observed. Instead, small lumps of condensed DPPC molecules were observed on the surfaces and the edges of the OTS islands. The surface of the DPPC monolayer on the SiO@sub 2@ area was almost the same height as the OTS island surface. These results indicate that the OTS SAM island has a potential of effective anchor molecules in DPPC BLM depositions on SiO@sub 2@ surfaces.

BI-WeP5 Characterization and Durability of Organosilane Self-assembled Monolayers on the Native Titanium Oxide Surface, ***R.M. Lennen***, *R.A. Brizzolara,* NSWC, Carderock Division

Titanium is a common material of heat exchangers and seawater piping systems on U.S. Naval vessels, as well as a key biomedical implant material. Several different organosiloxane self-assembled monolayers (SAMs) have been prepared on cleaned and hydroxylated titanium surfaces and characterized with x-ray photoelectron spectroscopy (XPS), angle-resolved XPS, and contact angle measurements. Precursors include trichlorosilanes and trialkoxysilanes with a wide array of terminal functional groups. Perfluorinated SAMs and multilayers were tested for their durability in natural filtered seawater from Port Everglades, FL; artificial seawater; artificial seawater inoculated with the biofilm forming bacterium Deleya marina; and flowing seawater at Port Everglades under two flow velocities. The thermal stability of coatings formed from alkyltrialkoxysilane precursors on titanium was also investigated in ultrahigh vacuum. In the future, these self-assembled monolayers will be used to investigate biofilm adhesion as a function of critical surface tension. This will lead to the development of ultra-thin antifouling coatings for shipboard titanium heat exchanger tubes with seawater intake. This work was funded by the NSWC Carderock Division In-House Laboratory Independent Research program and the Office of Naval Research.

BI-WeP6 Amine-Reactive Mixed Monolayers on Scribed Silicon with Controlled Levels of Functionality: The Reaction of Scribed Silicon with Epoxides, ***M.R. Linford***, *Y.-Y. Lua,* Brigham Young University

Epoxides are important in industry and in bioconjugate chemistry because of their reactivity with amines, sulfhydryls, and other nucleophiles. Here we report a significant advance in the preparation of patterned and functionalized silicon surfaces by showing that epoxides readily react with scribed silicon to yield monolayers with even greater efficiency that was reported for 1-alkenes,@footnote 1@ 1-alkynes,@footnote 1@ 1-haloalkanes,@footnote 2@ and alcohols.@footnote 3@ Mixed monolayers were prepared from solutions of 1,2-epoxyoctane and 1,2,7,8-diepoxyoctane to control the number of free epoxide groups at the surface. The amine reactivity of these surfaces increases as the fraction of 1,2,7,8-diepoxyoctane in the monolayers increases. The formation of monolayers occurrs by wetting a dry, oxide-coated or hydrogen-terminated silicon surface with a liquid epoxide or Î±,Ï‰-diepoxide and by scribing in the air with a diamond-tipped instrument or tungsten carbide ball. In addition to this fundamental work, we plan to discuss i) the formation of biotinylated surfaces through a reaction of epoxide surfaces with biocytin (a lysine-

biotin complex) and DNA-containing surfaces (through a reaction with amine-terminated oligonucleotides), and ii) patterning of silicon with these coatings using an AFM tip.@footnote 4@ @FootnoteText@ @footnote 1@ Niederhauser, T.L.; Jiang, G.; Lua, Y.-Y.; Dorff, M.; Woolley, A.T.; Asplund, M.C.; Berges, D.A.; Linford, M.R. Langmuir 2001, 17, 5889-5900.@footnote 2@ Niederhauser, T.L.; Lua, Y.-Y.; Sun, Y.; Jiang, G.; Strossman, G.S.; Pianetta, P.; Linford, M.R. Chemistry of Materials 2002, 14, 27-29.@footnote 3@ Niederhauser, T.L.; Lua, Y.-Y.; Jiang, G.; Davis, S.D.; Matheson, R.; Hess, D.A.; Mowat, I.A.; Linford, M.R. Angew. Chem. Int. Ed. 2002, 41(13), 2353-2356.@footnote 4@ Wacaser, B.A.; Maughan, M.J.; Mowat, I.A.; Niederhauser, T.L.; Linford, M.R.; Davis, R.C. Applied Physics Letters 2003, 82(5), 808-810.

BI-WeP7 Molecular Engineering of Surfaces for Sensing and Detection, *C.L. Boozer, J. Ladd, A. Taylor,* ***Q. Yu****, J. Homola, S. Jiang,* University of Washington

There is an urgent demand for developing sensors capable of quantitative and simultaneous detection, identification, and monitoring of multiple analytes in complex media for various applications ranging from homeland security and medical diagnostics to food and environmental monitoring. Immunological detection with antibodies is perhaps the only technology that has been successfully employed for the detection of bacteria, viruses, proteins, and low-molecular-weight compounds. In this talk, we will discuss our recent effort on molecular engineering of surfaces for sensing and detection. First of all, control of antibody orientation is achieved on charged surface assembled monolayers (SAMs). Antigen is used to probe antibody orientation measured by surface plasmon resonance (SPR) biosensor while direct evidence of preferred antibody orientation is provided by the time-of-flight secondary ion mass spectrometry. Second, it was shown in our previous work that the behavior of protein adsorption depended on nano-scale structures of a surface with which proteins interact. Polyethylene glycol (PEG) SAMs are used as a model surface to study surface resistance to protein adsorption. Atomic force microscopy/scanning tunneling microscopy (AFM/STM), SPR and molecular dynamics simulation techniques are used in such studies. Results show light on molecular-level understanding of non-fouling mechanism. Third, a new DNA-based protein immobilization method has been developed for use with SPR biosensors. This DNA-based immobilization method provides a convenient and versatile for multi-channel biosensors. We will demonstrate the quantitative and simultaneous detection of various analytes ranging from larger-sized to small-molecular weight analytes (e.g., E. coli, SEB, and simazine) in complex matrices (e.g., milk and ground beef) based on this new platform. Finally, we achieved single-molecular detection of immunoreactions using an AFM-based sensor.

BI-WeP9 Multilayers of Functionalized Liposomes for Improved SPR Analysis of Transmembrane Proteins, ***A. Granéli,*** *F. Höök,* Chalmers University of Technology, Sweden

The cell membrane consists of a large fraction of transmembrane proteins, which mediates and performs a large number of reactions taking place in the cell membrane or at the cell membrane surface. All transmembrane proteins consist of a hydrophobic part that transverse the bilayer, which make most of them insoluble in water and therefore difficult to study. Accordingly, functional studies of individual transmembrane proteins generally require dissolving or reconstituting procedures, such as the use of detergents or incorporation in lipid assemblies such as proteoliposomes. In biosensing applications, including drug screening and medical diagnostics, as well as for fundamental studies of transmembrane proteins, surface-based techniques have turned out to be important analytical tools. Application of such techniques require that the proteins are immobilized on a solid surface, which often tends to have a negative influence on the protein activity. The necessity of having the transmembrane proteins residing in lipid membranes complicates immobilization of sufficient amounts of protein. To allow the use of surface analytical tools for studies of transmembrane proteins, protocols that enhance the amount of immobilized protein, thus the signal, are required. For that purpose, we have developed a strategy where multilayers of proteoliposomes are immobilized on Au or SiO@sub 2@ surfaces, proven versatile for studies of ligand-interaction kinetics using the quartz crystal microbalance with dissipation monitoring (QCM-D) and surface plasmon resonance (SPR) techniques. This was achieved by utilizing a DNA-modified surface, to which proteoliposomes modified with complementary DNA was immobilized; a process that was possible to repeat up to at least 6 layers, thus allowing the use of the full sensing depth of QCM-D and SPR. Signal-amplification using the liposome multilayer approach was proven via dissociation or binding from/to the transmembrane protein transhydrogenase.

BI-WeP10 Surface Physico-Chemical Studies of Immobilised Oligonucleotides, ***P-C.T. Nguyen****, S. Kumar,* University of South Australia, Australia*; M. DeNichilo,* TGR BioSciences, Australia*; N. Voelcker,* Flinders University of South Australia, Australia*; H.J. Griesser,* University of South Australia, Australia

Single-stranded oligonucleotides can bind nucleic acid targets as well as other targets such as small molecules, peptides, proteins and cells. Compared to antibodies, the selectivity, specificity and affinity of oligonucleotides are equal and often superior. Thus, surface-immobilised oligonucleotides have become attractive choices as recognition elements in microarrays for high throughput, parallel and multidimensional analysis in biomedical diagnostics, and aptamers are increasingly replacing antibodies as molecular recognition elements. So far, most of the research involving oligonucleotide probes has focussed on end applications for the life sciences, with some work on fundamental aspects of surface immobilisation and target binding to immobilised oligonucleotides. In order to harness the apparent power of such arrays, a more detailed physical and chemical understanding is required, in addition to optimising the immobilisation process. Contributing factors include substrate, oligonucleotide structure, immobilisation chemistry and surface density of immobilised oligonucleotides. Our focus is to characterise and optimise the density of immobilised oligonucleotides, and measure hybridisation efficiency for a specific choice of substrate, oligonucleotide, and immobilisation chemistry. Glass and silicon are the most commonly used substrates but we are extending immobilisation to polymeric carriers. XPS, ToF SIMS and AFM are used to surface characterise the substrate and attached oligonucleotides. Using principles of surface science we study molecular interactions between oligonucleotide chains, and the effects that the structure and packing density of the oligonucleotide coating have on hybridisation, assessed by MALDI-ToF-MS. It is expected that oligonucleotide density will have a direct bearing on activity and steric availability for hybridisation.

BI-WeP11 Characterization of DNA Microarrays, ***D. Barbash****, J.E. Fulghum, Y. Wu, G.P. Lopez,* University of New Mexico

DNA microarrays are widely used for gene expression studies. Production of DNA microarrays includes attachment of single stranded DNA or oligonucleotides onto a variety of different substrata. Methods that are used include directed synthesis of oligonucleotides by photolithography and printing pre-existing cDNA using precision robots. Substrates available for immobilization are gold, modified glasses (aminosilane or polylisine) and filter membranes. There are multiple functional groups in DNA that are capable of attachment the surface to the substrate. The purpose of our study is to reveal the chemistry behind DNA attachment to surfaces. We are using the ATMS(p-aminophenyl trimethoxy silane)/diazotization method to spot oligonucleotides on a microscope glass surface.(Dolan, P.L. et al. Nucleic Acids Research 2001, 29, 21e107). This method include immersion of cleaned microscope glass into the ATMS solution, its activation by NaCl and HCl and spotting DNA onto it. The method results in robust covalent attachment of the DNA in a manner that is compatible with subsequent hybridization.The methods for studying attached nucleotides include X-ray Photoelectron Spectroscopy (XPS) and Attenuated Total Reflection Spectroscopy (ATR-FTIR). XPS allow us characterize the surface composition from less than 10nm depth while ATR-FTIR technique provides chemical information from up to 1mm of the surface.

BI-WeP12 DNA-Based Protein Immobilization vs. Biotin/Streptavidin Bridges, ***C.L. Boozer****, J. Ladd, Q. Yu, S. Chen,* University of Washington*; J. Homola,* Institute of Radio Engineering and Electronics, Czech Republic*; S. Jiang,* University of Washington

A new DNA-based protein immobilization method has been developed for use with SPR biosensors. This DNA-based immobilization method provides a convenient and versatile alternative to the commonly used biotin/streptavidin platform, with comparable, if not better, sensitivity. This work presents a comparison of these two platforms, focusing on the detection of hCG as a model system. Our results show that the DNA-based method allows for detection of lower hCG concentrations. Extensive control experiments have been performed to check both sensor platforms for non-specific binding and cross reactivity. In addition to the increased sensitivity, the DNA-based protein immobilization offers many other advantages crucial to biosensor development that the biotin/streptavidin platform does not have. While both the biotin/streptavidin complex and the DNA-based approach are robust and highly specific, the DNA based approach is much more versatile.

BI-WeP13 Molecular Simulation of Mixed SAMs Including Thiolated DNAs on Gold (111) Surfaces, ***J.P. Sullivan***, *S. Jiang,* University of Washington

The ability to tether DNAs to a solid support has yielded a variety of practical technologies including DNA microarrays and DNA based biosensors. Yet in spite of the rapid advances of surface tethered DNAs in biotechnological applications, improvements to these technologies are made through a painstaking combinatorial process that suffers from a lack of mechanistic understanding. It has been shown that the hybridization of ssDNA SAMs can be affected by the introduction of a non-DNA terminated thiol as a diluent. Experimentalists in our group, for example, are using oligo-ethylene glycol (OEG) terminated thiols along with thiolated ssDNA to form mixed SAMs on gold (111). Speculation has been unable to yield a predictive tool for which diluent length and density will have the best impact on a given DNA probe. Accordingly, we turned to simulation to provide atomic resolution images of these mixed SAMs, revealing information that could not be intuited. All simulations were carried out in explicit solvent with Na+ to balance charge, and NaCl to control ionic strength. The CHARMM27 all-atom potential force field was used to model the DNAs, while the TIP3P potential was used for water interactions. The OEGs were treated using a SJY force field with demonstrated accuracy for OEG-thiols. We present results for the packing of pure DNA SAMs (both single and double stranded, of varying sequence lengths and compositions, and at different ionic strengths). The pure SAM packing results were then used to set up simulations of DNA SAMs mixed with oligo-ethylene glycol at varying diluent lengths (number of repeat units) and densities, for which results are also reported. These results will be instrumental in developing theory-based methods for selecting diluents and diluent densities. This will reduce or eliminate the trial and error process involved in determining diluent properties for the countless possible DNA probes that do not already have optimized diluents.

BI-WeP14 Vacuum-Based Diagnostics of Aqueous Microenvironments Using Evaporative Micro-Orifice Technique, ***T.M. Valentine***, *J.J. Park, G.W. Rubloff,* University of Maryland

While gas and surface chemical analysis techniques can be applied to aqueous systems (e.g., electrospray mass spectrometry), the high surface/volume ratio of bioMEMS environments places a premium on biochemical characterization directly at or within the microfluidic system. We are exploring the direct sampling of the aqueous microenvironment via a micron-scale evaporative orifice which couples the microfluidic system to vacuum-based chemical analytical tools. Considering a variety of coupling designs, simulations indicate the possibility to observe volatile species (dissolved gases such as O@sub 2@, CO@sub 2@, and VOC's), metabolic activity of microorganisms, and nonvolatile species ejected as a consequence of microfluid dynamics at the sampling orifice. For orifice sizes up to 30 µm, differential pumping by the vacuum system will maintain sufficiently low pressures for operation of the vacuum analysis instruments. Considering the large water background and typical mass spectrometry sensitivity (200 ppb), simulations indicate that signals should be measurable from bacterial CO@sub 2@ and VOC evolution. Given flow rates 1-100nL/min at the orifice, ejection and measurement of nonvolatile organic species (proteins, biopolymers) should be possible at concentrations of biological interest. An experimental testbed has been developed to integrate aqueous environments with appropriate vacuum sensing equipment. Results of testing the experimental setup under various conditions, confirming and calibrating the simulation, and expanding the evaporative-orifice concept to integrate microfluidic devices being developed in parallel will be discussed. This effort was undertaken as a senior thesis project with the assistance of a 2003 AVS Undergraduate Research Award.

BI-WeP15 Effects of Surface Treatment and Curing Conditions on Poly(Dimethylsiloxane) Metallization for Retinal Prosthesis, ***M. Maghribi***, *C. Evans, K.J. Wu, A.J. Nelson,* Lawrence Livermore National Laboratory

Surface properties have a critical impact on the general performance of polymers and elastomers. Surface contamination, such as siloxane surfactants, can alter the surface properties of the material thus affecting the fabrication processes. Inadequately cured poly(dimethylsiloxane) (PDMS) is highly mobile and can cause adhesion failures. In this work we explore how surface treatments and PDMS cure time impacts process development for hybrid retinal implants. For example, oxygen plasma treatment is used to promote wetting of the PDMS surface as well as promoting adhesion. To photolithographically pattern metal traces on PDMS is not a trivial task and fundamental material characteristics must be examined to develop reliable and repeatable fabrication processes. Time of flight secondary ion mass spectrometry (ToF-SIMS) and high resolution X-ray photoemission spectroscopy (XPS) were utilized to reveal the surface chemistry attributed to different surface treatments and curing conditions. ToF SIMS results indicate that the basic molecular and chemical structure of poly(dimethylsiloxane) is altered under O@sub 2@ treatment. Specifically, a strong oxidation reaction to the dimethlysiloxane group occurs, replacing methyl with silanol groups; which is ultimately responsible for the success in metallization. XPS quantitative analysis revealed an oxygen rich surface with significantly increased Si-O bonding. In addition, high-resolution C 1s, O 1s and Si 2p core-level spectra revealed additional C-O and O-Si-O bonding following O@sub 2@ plasma treatment. We conclude from these results that the explanation for the affinity of metals to adhere to the PDMS following O@sub 2@ plasma treatment is due to the reactive Si-O group formed on the surface. This work was performed under the auspices of the U.S. Dept. of Energy by the University of California Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48.

BI-WeP16 Bioactivity of Titanium Coatings Prepared by Reactive Plasma Spraying, ***M. Inagaki***, *Y. Yokogawa, T. Kameyama,* National Institute of Advanced Industrial Science and Technology (AIST), Japan

A simple treatment method using radio-frequency reactive plasma spraying (rf-RPS) was studied to induce bioactivity of titanium (Ti) coatings. Ti coatings were deposited on Ti substrates by a rf-RPS method using a thermal plasma of Ar gas containing 1-6% N@sub 2@ and/or O@sub 2@ at a input power of 16 kW. Ti powders impregnated with calcium were also sprayed. Composition change of coating's surface during soaked in a simulated body fluid (SBF)@footnote 1@ was examined by micro Fourier transform infrared spectroscopy and thin film X-ray diffraction. Ti coatings prepared with Ar-O@sub 2@ and Ar-N@sub 2@-O@sub 2@ plasma formed apatite after 3 days of soaking in 40 ml SBF. This indicates that such coatings have the ability to form a biologically active bone-like apatite layer on the surface. In the XRD patterns for both Ti coatings, minute peaks ascribable to TiO@sub 2@ (anatase and rutile phase) were commonly observed. On the other hand, composition change of coating's surface cannot be observed for Ti coating sprayed with pure Ar and Ar-N@sub 2@ plasma after 7 days of soaking in SBF. The 0.05-0.2 mol% impregnated Ti coatings prepared with Ar-O@sub 2@ and Ar-N@sub 2@-O@sub 2@ plasma formed apatite after 7 days of soaking in SBF. Thus it seems that calcium impregnation into Ti powders somewhat inhibited to form apatite at surface of coatings. Ti coatings with Ar-N@sub 2@-O@sub 2@ plasma gave excellent adhesion to substrate, whereas Ti coatings with Ar-O@sub 2@ plasma gave poor adhesion. Therefore, surface modification of Ti splat by Ar-N@sub 2@-O@sub 2@ plasma is an effective method to provide excellent adhesion and bioactivity for plasma sprayed Ti coatings. @FootnoteText@ @footnote 1@HM Kim, F. Miyaji, T Kokubo, T Nakamura, J. Biomed. Mater. Res 45, (1999)100-107.

BI-WeP17 RF Plasma Deposition of Acrylic Acid Thin Films: Relationship between Plasma Charaterisation and Films Physicochemical Properties, *N. Rossini, A. Valsesia, G. Ceccone, P. Colpo,* ***F. Rossi***, Joint Research Centre, Italy

Acrylic acid thin films have been deposited by continuous and pulsed RF capacitive discharge. In situ diagnostics (Langmuir probe, Mass spectrometry and Self Excited Electron Resonance Spectroscopy) are used for the different plasma conditions to analyse the framentation processes and identify the species contributing to the growth of the film. The composition and physico chemical properties of the films are analysed with FTIR, XPS, contact angle and Quartz Crystal Microbalance with Dispersive mode. Relationship with plasma characterisation is established. Experimental conditions leading to high concentration of COOH functionalities as well as films stability are determined.

Biomaterial Interfaces
Room 307 - Session BI+SS-WeA

Biomolecular Surface Science and Microfluidics
Moderator: M. Grunze, Universität Heidelberg, Germany

2:00pm **BI+SS-WeA1 Soft X-ray SpectroMicroscopy of Bio-interfaces***, A.P. Hitchcock, C. Morin, T. Araki, J.L. Brash, R. Cornelius,* McMaster University, Canada*; S.G. Urquhart, U.D. Lanke,* University of Saskatchewan, Canada*; N. Samuel, D.G. Castner,* University of Washington **INVITED**

We are using scanning transmission x-ray microscopy (STXM) and X-ray photoemission electron microscopy (X-PEEM) to study the adsorption of biological and bio-active species on naturally and artificially patterned polymer and molecular substrates. In one area, the thrust is to investigate fundamental issues of protein - polymer interactions relevant to blood compatibility. In a second area, we are providing feedback for the development of patterned bio-active surfaces. Both synchrotron-based microscopies provide chemical speciation by near edge X-ray absorption spectroscopy (NEXAFS). STXM provides quantitative chemical mapping at a spatial resolution of 50 nm, with the possibility of detecting proteins on polymer thin films with monolayer sensitivity in the presence of an aqueous overlayer of the protein solution.@footnote 1@ X-PEEM has greater surface sensitivity than STXM but requires placing the sample in vacuum. Our X-PEEM studies of fibrinogen adsorption on a model polymer substrate, phase segregated polystyrene-poly(methylmethacrlyate) (PS/PMMA),@footnote 2@ indicate clear preference for adsorption on the PS domains when adsorption is performed from a phosphate buffer solution, but preference for adsorption at the PS-PMMA interface when using non-buffered solutions. The strengths, limitations, and future potential of soft X-ray microscopy for studies of bio-interfaces will be discussed.@footnote 3@ @FootnoteText@ @footnote 1@ A.P. Hitchcock et al., J. Biomaterials Science, Polymer Ed. 13 (2002) 919.@footnote 2@ C. Morin et al, J. Electron Spectroscopy 121 (2001) 203.@footnote 3@ X-ray microscopy carried out at the Advanced Light Source (supported by DoE under contract DE-AC03-76SF00098) and the Synchrotron Radiation Centre (supported by NSF under award DMR-0084402). Research supported financially by NSERC (Canada) and the Canada Research Chair Program. We thank the PEEM-2 staff (A. Scholl, A. Doran) for assistance in these studies.

2:40pm **BI+SS-WeA3 Interaction of Protein Solutions with Biocompatible Organic Monolayers: An In Situ Neutron Reflectometry Study***, R. Dahint, D. Schwendel,* University of Heidelberg, Germany*; F. Schreiber,* University of Oxford, UK*; M. Grunze,* University of Heidelberg, Germany

Oligo(ethylene glycol) (OEG) terminated self-assembled monolayers (SAMs) effectively prevent the adsorption of proteins from biological solutions. Yet, efforts are still being made to elucidate the mechanisms of protein resistance on a molecular level. For proteins deposited on the tip of an atomic force microscope (AFM), long range repulsive forces have been observed upon approaching protein resistant methoxy-terminated tri(ethylene glycol) undecanethiolate SAMs (EG3-OMe) on gold.@footnote 1@ However, as proteins adsorbed on the tip may undergo significant structural changes, it is not obvious that the same strength and type of interaction is experienced by freely moving, dissolved molecules. We, therefore, used neutron reflectometry to investigate protein/surface interactions employing biomolecules in their native state and natural environment. Room temperature measurements on protein resistant films of EG3-OMe in contact with bovine serum albumin (BSA) solutions reveal the presence of an extended protein depletion layer with a thickness of about 50 nm between the SAM and the bulk protein solution. The results are compared to the strength and range of repulsive forces measured by AFM. Temperature dependent studies on the EG3-OMe/water interface reveal, that a previously observed, density reduced water phase in the vicinity of the SAM cannot account for the protein resistant properties of the films. @FootnoteText@@footnote 1@ K. Feldman, G. Hähner, N. D. Spencer, P. Harder, M. Grunze, J. Am. Chem. Soc. 1999, 121, 10134.

3:00pm **BI+SS-WeA4 Characterization of Lipid Bilayers on Functionalized Surfaces***, T.W. McBee, S. Saavedra,* University of Arizona

The utilization of planar supported lipid bilayers (PSLBs) for technological applications is limited by their fragility. They can be destroyed by a variety of conditions, including exposure to air, surfactants, and mechanical stress such as elevated temperatures. One way to overcome this limitation is to introduce polymerizable groups into the tail region of the lipid molecules and stabilize the bilayer structure through polymerization, which results in a very stable film when formed on silica. We have been investigating the characteristics of lipid bilayers, both polymerized and unpolymerized, on a variety of functionalized surfaces, including self-assembled monolayers (SAMs) as well as self-assembled polymer multilayers compared to bilayers formed on silica. This talk will focus on our investigations of PSLBs on Î³-aminopropyl silane (GAPS) monolayers as well as on mixtures of polyaniline/poly(acrylic acid). These types of surfaces are of interest due to their potential for energy transduction and sensing applications.

3:20pm **BI+SS-WeA5 Intact Vesicle Adsorption and Supported Biomembrane Formation from Vesicles in Solution Studied by a Combined SPR and QCM-D Instrument, and AFM***, E. Reimhult*[1]*, F. Höök, B. Kasemo,* Chalmers University of Technology, Sweden

Two biomembrane model systems receiving high interest are unilamellar phospholipid vesicles and supported planar phospholipid bilayers. We have investigated the adsorption kinetics of small unilamellar POPC vesicles with a setup, combining the Quartz Crystal Microbalance with Dissipation technique and Surface Plasmon Resonance techniques in parallel. Using this instrument we have for the first time simultaneously and in real time measured the acoustic (hydrated) and optical (dry) mass for vesicle adsorption on SiO@sub 2@ and oxidized Au. These measurements have significantly extended our understanding of intact vesicle adsorption on surfaces, as a function of surface chemistry,@footnote 1@ temperature,@footnote 1,2@ vesicle size,@footnote 3@ and osmotic stress.@footnote 1@ We have been able to distinguish between the mass response due to adsorbing intact vesicles and that of planar bilayers formed on the surface via vesicle rupture. Furthermore, we have; determined the critical coverage required for vesicle rupture on SiO@sub 2@, obtained a measure of the amount of trapped water and deformation of intact vesicles on the surface, studied the kinetics of the final part of the supported planar bilayer formation process on SiO@sub 2@ and detected loss of lipids on its completion. The latter two are critical for the quality of the formed bilayer, i.e. limiting the number of defects. We also intend to present results from atomic force microscopy studies of vesicle adsorption on heterogenous (patterned) surfaces, with focus on the behavior at phase boundaries, separating planar bilayer forming and intact vesicle adsorbing areas. @FootnoteText@ @footnote 1@ E. Reimhult, F. Höök, and B. Kasemo, Langmuir 19, 1681 (2003)@footnote 2@ E. Reimhult, F. Höök, and B. Kasemo, PRE 66, 051905 (2002).@footnote 3@ E. Reimhult, F. Höök, and B. Kasemo, JCP 117, 7401 (2002).

3:40pm **BI+SS-WeA6 Microfluidic Systems for Applications in Chemistry and Biochemistry***, A. Manz,* Imperial College, UK **INVITED**

Fluid handling integrated into microsystems has been in use now for a number of years. Mostly, research has focused on micro pumps, valves, sensor flow cells and electrophoresis.@footnote 1,2,3@ However, the underlying idea of shrinking the whole analytical chemistry or biochemistry lab down to chip size will make it necessary to talk about interfacing these modules properly and efficiently. Recently, we have proposed a concept for a chemical microprocessor.@footnote 4,5@ This concept is related to an earlier attempt to define an analytical chemistry microsystem, micro-TAS (miniaturised total analysis system).@footnote 6@ Scaling laws predict 100x faster mass and heat transport, if a known system is miniaturized by a factor 10 (linear). This is particularly useful in capillary electrophoresis, chromatography and continuous-flow reactors. We have presented several examples in the past few years. I plan to show a few examples of recent chip developments taken from my lab: A horseradish peroxidase assay (400ms incubation), isoelectric focusing of a peptide in continuous flow (100x higher concentration in 300ms focusing time) and an air monitor based on a plasma emission chip. @FootnoteText@ references @footnote 1@Micro Total Analysis Systems 2000, A.van den Berg, W.Olthuis, P.Bergveld, eds., Kluwer Academic Press, ISBN 0-7923-6387-6 (2000). @footnote 2@D.R.Reyes, D.Iossifidis, P.A.Auroux, A.Manz, Anal Chem 74, 2623-2636 (2002) @footnote 3@P.A.Auroux, D.Iossifidis, D.Reyes, A.Manz, Anal Chem 74, 2637-2652 (2002) @footnote 4@A.Manz, H.Becker, Transducers 97, Chicago, June 16-19, 1997, Digest of technical papers (1997) 915-918. @footnote 5@M.C.Mitchell, V.Spikmans, A.Manz, A.J.de Mello, J.Chem.Soc., Perkin Trans.1 2001 (2001) 514-518. @footnote 6@A.Manz, N.Graber, H.M.Widmer, Sens. Actuators B1 (1990) 244-248.

4:20pm **BI+SS-WeA8 Interfacial Engineering for Protein Biochips in Proteomics Applications***, H. Lu, P. Kernen, P. Wagner,* Zyomyx, Inc.

Proteomics is increasingly dependent on analytical tools that focus on quantification of protein expression, biomolecular-protein interactions, and

[1] **Morton S. Traum Award Finalist**

functional activity. The large numbers of proteins and complexity involved in proteomics applications present tremendous challenges for the development of analytical platforms and specifically interfacial engineering schemes. Zyomyx has developed a novel protein biochip platform that facilitates rapid, precise, highly multiplexed analysis with minimal sample requirements and has integrated several sophisticated interfacial engineering strategies. The biochip architecture consists of a three-dimensional array structure designed to provide consistent feature size and defined placement, while eliminating spot-to-spot cross contamination. We will focus on details of biochip development with an emphasis on organic layer compositions for optimal packing density, molecular orientation, selective immobilization of capture reagents, and low non-specific protein adsorption. Examples on high-level quantitative protein analysis will focus on Zyomyx human Cytokine Biochip capable of fully multiplexed and quantitative protein analysis based on sandwich-immunoassay configurations.

4:40pm **BI+SS-WeA9 Arrays of DNA-tagged Vesicles Based on Spontaneous Sorting to a DNA-array Template**, ***I. Pfeiffer***, *S. Svedhem, F. Höök,* Chalmers University of Technology, Sweden

We have developed a surface-modification protocol that allows sorting of DNA-tagged vesicles (where DNA is anchored via a cholesterol moiety) to arrays of cDNA-modified Au-spots on a SiO@sub 2@ surface. Biotinylated albumin (biotin-BSA) was chosen to functionalize Au spots surrounded by SiO@sub 2@, while supported phospholipids bilayers was formed on the surrounding SiO@sub 2@, thus representing an inert background. This allowed subsequent coupling of biotinylated DNA strands via neutravidin bound to biotin-BSA only on Au spots. Eventually, a cDNA array created in this way was proven compatible with specific immobilization of differently DNA-tagged vesicles utilizing complementary DNA hybridization. The surface functionalization protocol was established using the quartz crystal microbalance technique with dissipation monitoring (QCM-D), allowing quantification of the different immobilization steps, while fluorescence microscopy was used to analyze the vesicle sorting. The compatibility of the vesicles to act as carriers for proteins was proven using scFv-antibodies, anchored to the DNA-tagged vesicles via nitrilotriacetic acid (NTA)-functionalized lipids. These results thus open up the prospect to use surface directed sorting of functionalized vesicles for construction of protein arrays, avoiding complicating and/or destructive microfluidics or microdispensing protocols. The work also includes a number of alternative approaches towards the creation of DNA arrays being compatible with the above described principle for spontaneous vesicle sorting, including improvements in the strength of the cholesterol-DNA based coupling and the dimension of the arrays, which has the potential to be down-scaled to the length of the DNA probes and the size of the vesicles, typically being less than 100 nm.

5:00pm **BI+SS-WeA10 Immobilization of Oriented Protein Molecules on High-density Poly(ethylene glycol) Coated Si(111)**, ***T. Cha,*** University of Minnesota*; A. Guo,* MicroSurfaces, Inc.*; X.-Y. Zhu,* University of Minnesota

The success of DNA microarray technology has motivated the development of similar tools for proteins. One of the key challenges in this chip-based assay is how the liquid-solid interface is engineered to minimize nonspecific adsorption, to control protein conformation and orientation, and to present high specificity for protein attachment. We demonstrate the synthesis of high density poly(ethylene glycol)-coated Si(111) and its application as an excellent substrate for protein microarray technology. The surface is obtained from the reaction of a multi(8)-armed PEG (mPEG) molecule with a chlorine terminated Si(111) surface to give a PEG film with thickness of 5.2 nm. Four out of the eight arms on each immobilized PEG molecule are accessible for linking to the chelating iminodiacetic acid (IDA) group which binds Cu2+ ions (2.7x10^13/cm2). The resulting Cu2+-IDA-mPEG-Si(111) surface is shown to specifically bind 6x-histidine-tagged protein molecules, including green fluorescent protein and sulfotransferase, without the need of pre-purification. In the case of 6xHis-GFP, this immobilization strategy can lead to a closely packed monolayer of protein molecules. Background tests show that the surface retains its inertness towards non-specific protein adsorption in the absence of either a poly-His tag on the protein molecule or metal ions on the surface. Both the inertness of the chemical surrounding and the controlled orientation contribute to an ideal environment for the immobilized protein molecule to retain its native conformation and reactivity. Enzyme activity for surface immobilized sulfotransferase are measured and compared to solution phase values. Such a kinetic experiment essentially extends a 2-dimensional array to the third dimension-time.

Biomaterial Interfaces
Room 318/319 - Session BI-ThM

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

9:00am **BI-ThM3 Simultaneous Electrochemical and Tapping Mode Imaging of Soft Biological Samples with AFM Tip Integrated Nanoelectrodes and Nanobiosensors**, ***A. Kueng***, *C. Kranz,* Georgia Institute of Technology*; A. Lugstein, E. Bertagnolli,* Vienna University of Technology, Austria*; B. Mizaikoff,* Georgia Institute of Technology

Recent developments in combined scanning probe techniques are aiming at complementary, simultaneously mapped information on physical and chemical surface properties with high spatial resolution. The integration of micro and nanoelectrodes into AFM tips using micromachining and focused ion beam (FIB) techniques recently described by our research group@footnote 1,2@ enables to simultaneously obtain laterally resolved electrochemical information at the sample surface during tapping mode AFM imaging. A defined geometry of an electroactive surface integrated above the very end of the original AFM tip allows direct correlation of the current signal and the topographical information. Hence, the functionality of scanning electrochemical microscopy (SECM) can be integrated into AFM. The presented technology enables integration of potentiometric electrodes or micro-/nanobiosensors providing simultaneous in-situ information on bioactive processes at the sample surface during AFM imaging. Due to the achieved current-independent positioning of the integrated electrode, biosensor functionality can be realized by modification of the electrode surface with an enzyme receptor, such as peroxidase or glucoseoxidase. Furthermore, bifunctional probes are applied to simultaneously image topographical and electrochemical properties of biologically active sample surfaces in AFM tapping mode. The activity of an oxidoreductase immobilized into a periodic micro-pattern of a soft polymer matrix is electrochemically detected during AFM imaging in tapping mode. For the first time specific detection of a molecular product resulting from enzymatic substrate conversion was obtained during AFM imaging. @FootnoteText@ @footnote 1@ C. Kranz, G. Friedbacher, B. Mizaikoff, A. Lugstein, J. Smoliner, E. Bertagnolli, Anal. Chem., 73, 2491-2500 (2001). @footnote 2@ A. Kueng, C. Kranz, B. Mizaikoff, A. Lugstein, E. Bertagnolli, Appl. Phys. Lett., 82, 1592-1594 (2003).

9:20am **BI-ThM4 Simultaneous Atomic Force Microscopy and Fluorescence Imaging of Supported Biomembranes**, ***A.R. Burns***, *J.M. Gaudioso,* Sandia National Laboratories

Lateral organization of lipids and proteins in membranes is critical to cellular signaling processes. Separately, fluorescence imaging and atomic force microscopy (AFM) are both effective ways to map structures in supported membranes. However, the ability to correlate information gathered from fluorescence imaging of labeled biomolecules and lipids with detailed lateral structures mapped out with AFM is highly advantageous. We discuss simultaneous AFM and submicron confocal fluorescence imaging of domain structures in model lipid bilayers. Lipids labeled by fluorescent probes either at the headgroups or tailgroups enable domain contrast in fluorescence imaging on the basis of partitioning between gel and disordered liquid phases. However, correlation with AFM topographic information reveals that they do not always faithfully report exact gel domain size or shape. Furthermore, we find that the fluorescence contrast decreases significantly with domain size, such that small domains observed with AFM are not observed in fluorescence images despite adequate optical resolution. Imaging of labeled proteins bound to membrane receptors is also discussed. In all cases, the complete correlation of topographic and fluorescence images provides evidence that gel-phase domains occur across both leaflets of the bilayer. This research was supported in part by the Division of Materials Science and Engineering, Office of Basic Energy Sciences, U.S. Department of Energy. Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the U.S. Department of Energy under Contract DE-AC04-94AL85000.

9:40am **BI-ThM5 Force Spectroscopy Investigation of HIV Envelope Glycoprotein and Dual Antibody Complex using Atomic Force Microscopy**, ***Y. Lam***, Duke Univ. and Center for Biomolecular and Tissue Eng.*; W.K. Lee,* Duke Univ.*; P. Marszalek,* Duke Univ. and Center for Biomolecular and Tissue Eng.*; M. Alam,* Human Vaccine Institute*; R. Clark,* Duke Univ.*; B. Haynes,* Human Vaccine Institute*; S. Zauscher,* Duke Univ. and Center for Biomolecular and Tissue Eng.

Understanding the structure-function relationships of pathogenic molecules is the key to designing sensitive detection mechanisms, as well as effective inhibitory drugs. Atomic force microscopy (AFM) is an optimal tool for investigation of these molecular scale biomechanics, as it provides high temporal and spatial resolution while maintaining an aqueous testing environment. In this study, we use the AFM to examine a well-characterized model system of Human Immunodeficiency Virus-1 (HIV-1) envelope glycoprotein gp120 and several monoclonal antibodies. Antibodies were screened before AFM experiments using surface plasmon resonance (SPR), and chosen for greatest binding affinities. In the AFM experiment, one antibody, a human T-cell CD4 mimic, is immobilized on the surface, and functions to bind gp120 from solution. The second antibody, a human chemokine receptor mimic, is attached to the AFM tip, and only interacts with the bound gp120-CD4 complex. A large majority of trials registered adhesion events, qualitatively signifying the presence of gp120. Quantitative analysis determined antibody-antigen binding strengths on the order of 100 pN, in agreement with binding forces of other molecular recognition systems. Experiments with poly(ethylene glycol) tethers and variable pulling rates provide force profiles revealing details of attachment and detachment mechanisms. Results from this study show that AFM can be used effectively as a detection as well as characterization method to better understand the pathogenic system.

10:00am **BI-ThM6 Multi-technique Studies of Bio-Interface Processes; QCM-D, (Nanoparticle) SPR, SERS, AFM, Electrical Impedance, and Cell Force Sensor**, ***B. Kasemo***, Chalmers University of Technology and Goteborg University, Sweden **INVITED**

Title: Multi-technique studies of Bio-Interface kinetics with QCM-D,(nanoparticle) SPR, SERS, AFM, Electrical Impedance, and Cell Force Sensor. Text: Bio-interface sensing is commonly based on immobilization of some sensing molecules on a surface, and detection of how unknown sample molecules bind specifically to them. Techniques for detection include optical, electrical, mechanical and electro-acoustic methods. The first part of this talk describes the preparation of a sensing platform based on functionalized supported phospholipid bilayers, using QCM-D, AFM, SPR and electrical impedance (ac forward transmission factor).@footnote 1, 2@ Sensing applications with this platform are illustrated by several examples;@footnote 3@ DNA immobilization and hybridization, protein-bilayer interactions, and enzymatic reactions. With the QCM-D technique rich information is obtained through frequency and dissipation shift measurements at multiple (overtone) frequencies, additionally strengthened by combining this technique with SPR. The second part describes nano- and micro fabricated structures for sensing, including EBL fabricated Ag arrays for (G)SERS,@footnote 4@ colloidal lithography of nanoparticles for plasmon resonance enhancement,@footnote 5@ and standing cantilever arrays for cell force sensing.@footnote 6@ @FootnoteText@ @footnote 1@ Reimhult, E., et al., Langmuir 19 (2003) 1681-1691. @footnote 2@ Hook, F., et al., Analytical Chemistry 73 (2001) 5796-5804@footnote 3@ Hook, F., et al., Langmuir 17 (2001) 8305-8312. @footnote 4@ Gunnarsson, L., et al., NanoStructured Materials 12 (1999) 783-788.@footnote 5@ Hanarp, P., et al., Colloids and Surfaces A: Physicochemical and Engineering Aspects 214 (2003) 23-26@footnote6@ Petronis, S. et al Journal of Micromechanics and Microengineering (to be published).

10:40am **BI-ThM8 Coupling of his-tagged scFvs to Functionalized Lipid Assemblies for Array Based Sensing**, ***C. Larsson***, *F. Höök,* Chalmers University of Technology, Sweden

Lipid bilayers containing 5% NTA-lipids supported on SiO@sub 2@ have been used as a template for efficient immobilization of oligohistidine-tag containing single-chained antibody fragments (scFv) directed towards cholera toxin (CT). It was demonstrated that his-tagged scFv:s is equally efficient coupled to the NTA/Ni@super 2+@ containing lipid bilayer from a purified sample and the expression supernatant. Using the latter, time consuming protein purification steps is avoided. Independent on whether the coupling was made from the supernatant or from the purified sample, the template was proven efficient for antigen detection, in this case verified via the quartz crystal microbalance with dissipation monitoring

(QCM-D) technique using the antigen CT (Mw ~85 kD). Via a secondary amplification step utilizing G@sub M1@ containing vesicles, i.e. the membrane receptor for CT, sub-nanomolar concentrations of CT was detectable with QCM-D. Furthermore, this coupling strategy was also utilized for creation of protein array templates. The template was, however, based on novel DNA-array design, using streptavidin-based DNA-immobilization on gold spots, surrounded by a pure lipid bilayer on SiO@sub 2@, but with the aim to be used as a protein array rather than a DNA array. The latter was accomplished using DNA-modified lipid vesicles, directed to predefined DNA spots via complementary hybridization, where the protein-array concept was proven utilizing scFv-modified lipid vesicles utilizing NTA/Ni@super 2+@-based coupling for highly sensitive detection of fluorescently labeled CT.

11:00am **BI-ThM9 Real-Time, Quantitative Surface Plasmon Microscopy Measurements of Protein Adsorption**, ***J.S. Shumaker-Parry***, University of Washington, presently at the Max Planck Institute for Polymer Research, Germany*; M.H. Zareie, C.T. Campbell,* University of Washington

Surface plasmon resonance (SPR) spectroscopy has become a popular technique for measuring biomolecular interactions in real time with high sensitivity and without labels. SPR microscopy provides the same advantages as SPR spectroscopy with the added feature of using a CCD camera to image changes in reflected light intensity across a large area of a sensor surface simultaneously with good spatial resolution (~ 4 μm). Recently we have developed quantitative SPR microscopy methods for measurement of adsorption and desorption processes in real time based on monitoring changes in reflected intensity at a high contrast angle. For a small range of angles in a linear region of a SPR curve, reflectivity changes are proportional to effective refractive index changes near the sensor surface. By fixing the angle of measurement at a high contrast angle in such a linear region, refractive index changes may be monitored in real time by measuring reflectivity changes for pre-selected regions of a sensor surface. By extending methods used to quantitate SPR spectroscopy wavelength and angle shifts to changes in reflectivity measured by SPR microscopy, quantitative, real-time adsorption measurements are possible. We used these methods to measure adsorption of a DNA-binding protein to its DNA-binding site immobilized in a double-stranded DNA (dsDNA) array on a streptavidin linker layer to demonstrate the use of SPR microscopy for parallel, high-throughput array-based analysis. A major advantage of these array-based studies is the ability to use array elements without the DNA-binding site as reference regions to correct for non-specific adsorption and common refractive index changes. The real-time detection limit for fast time resolution measurements is less than ~8 x 10@super 6@ proteins per 200-μm array spot.

11:20am **BI-ThM10 Material and Surface Characterization of Electrodeposited Polysaccharide Chitosan Film as a Platform for Biomolecular Reactions in BioMEMS Systems**, ***C. Pederzolli***, *L. Lunelli, G. Speranza, R. Canteri, M. Anderle,* ITC-IRST, Italy*; J.J. Park, L.-Q. Wu, H. Yi, R. Ghodssi, W.E. Bentley, G.F. Payne, G.W. Rubloff,* University of Maryland

The polysaccharide chitosan provides a high density of amine sites for biomolecular adsorption and reaction. Exploiting the fact that under mildly acidic conditions (pKa=6.3) chitosan is water soluble with the amine groups positively charged, we have electrodeposited chitosan onto negative electrodes and demonstrated schemes for attachment of fluorophores, proteins and nucleic acids. The chitosan films can be deposited as relatively compact films or hydrogels, depending on process conditions. We have studied film properties by changing to high pH after deposition, rinsing, and drying. Measurements indicate a complex structure with density substantially lower than expected for a closed packed film, consistent with AFM images which reveal a morphology with substantial (50-150 nm) roughness. One may anticipate deprotonation and local enhancement of the pH at the surface during deposition, which could be accompanied by H@sub2@ evolution; localized features observed in AFM images could be consistent with H@sub2@ bubble formation during electrodeposition. Estimates of amine site density made from these observations are in agreement with fluorescence intensity measurements that directly reveal densities in the range 10@super14@-10@super15@ sites/cm@super2@. XPS and ToF-SIMS reveal chemical components of glucosamine (-CH@sub2@-NH@sub2@) and also of N-acetylglucosamine (-NH-(C=O)-) the monomeric residues of chitosan. The extent of cell adhesion on these chitosan films was analyzed using fibroblast-like cells (NIH-3T3, HGF-1), and results indicate that cell adhesion and growth are dependent on deposition parameters and film thickness. These observations of the materials and surface properties are important for our use of electrodeposited chitosan as a platform for biomolecular reactions in bioMEMS systems.

11:40am **BI-ThM11 Infrared and Visible Spectroscopy of Anisotropic Spin-cast Chitosan Films**, ***W.H. Nosal***, *S. Sarkar, A. Subramanian, D.W. Thompson, J.A. Woollam,* University of Nebraska, Lincoln

Chemical modification of chitosan is postulated as a way to control thrombosis at the surface of biomedical implants. Recent interest in performing surface modification of biomaterials has led to chitosan as a candidate to improve biocompatibility, due to the relative ease with which the amine groups can be chemically modified. As a way to evaluate surface modification effectiveness, spin-cast films of chitosan on silicon wafers were characterized using both infrared and visible spectroscopic ellipsometry (SE). Infrared SE data was modeled using a set of harmonic oscillators to represent the chemical bonds of the chitosan molecule. Visible SE data was modeled using an anisotropic uniaxial Cauchy dispersion model. In-plane vs. Out-of-plane optical anisotropy was detected in both the Infrared and visible wavelength data. This work shows there is a preferred orientation of the molecule, likely a result of spin casting. Visible ellipsometry surface mapping was used to determine anisotropy as a function of position on the wafer. This work discusses anisotropy and orientation of identified chemical bonds in a biomolecular film by spectroscopic ellipsometry.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Biomaterial Interfaces
Room 318/319 - Session BI+PS-FrM

Plasma Methods for Bio-interfaces

Moderator: E.R. Fisher, Colorado State University

8:20am **BI+PS-FrM1 Precision Chemical Control of Plasma Deposition for Smart Biosurfaces**, ***B.D. Ratner,*** University of Washington **INVITED**

In recent years, methods have evolved to deposit thin organic films from plasma environments that exhibit good control of chemistry along with the uniformity and substrate adhesion expected from plasma deposition. Three examples will be presented illustrating chemical control with special application to biomaterials. (1) Poly(N-isopropyl acrylamide) (pNIPAM) exhibits a solubility transition at 32 Â°C in an aqueous environment. When grafted onto a solid substrate, the pNIPAM phase transition produces a "smart" surface with strongly varying physical properties switchable with small temperature changes. Cells adhere and grow on ppNIPAM at 37Â°C and detach from the surface at room temperature. The ppNIPAM surfaces are non-toxic and excellent for cell growth. A microheater array can spatially control cell attachment to a ppNIPAM-treated chip. This suggests possibilities for cellomic and proteomic devices. (2) Since plasma environments destroy complex biomolecules, a new instrument has been constructed that combines electrospray ionization with plasma treatment of surfaces to produce a fast, efficient, flexible means to treat the surfaces of biomaterials with active biomolecules. The system has been successful in depositing intact, chemically bound hyaluronic acid (HA) onto plasma-activated stainless steel surfaces. (3) Poly(L-lactic acid) (PLLA) has been widely applied in tissue engineering scaffolds or for delivery of bioactive molecules, as it breaks down in the body to lactic acid, a component of the normal metabolism The pulsed plasma deposition techniques has been used to form thin PLLA coatings using cyclic lactide monomer. Such films degrade in a phosphate buffer solution.

9:00am **BI+PS-FrM3 Investigation of Organic Monomers in Plasma-induced Chemical Micropatterning**, ***G.Sh. Malkov,*** *M.L. Godek, D.W. Grainger, E.R. Fisher,* Colorado State University

Plasma-enhanced chemical vapor deposition (PE-CVD) of organic films is a valuable technique for the surface modifications of polymeric biomaterials. Recently, plasma-based methods have been developed for the fabrication of chemical micropatterns, which have a number of applications, including production of multianalyte biosensors, diagnostic tests, DNA microchips, and genomic arrays. The generation of micropatterns by means of the plasma deposition of organic compounds through a transmission electron microscope (TEM) grid mask has been reported.@footnote 1@ Here, we have created various high fidelity micron-scale patterns of different chemistries using inductively coupled pulsed RF plasma deposition through a TEM grid with the following monomers: acrylic acid, N-vinyl-2-pyrrolidinone, 2-hydroxyethyl methacrylate, N-vinylformamide, allylamine, and hexylamine on PS coated with plasma deposited, highly hydrophobic fluorocarbon materials. SEM images of the patterned surfaces will be demonstrated. Physico-chemical properties of deposited polymeric materials were characterized using angle-resolved XPS, FTIR, spectroscopic ellipsometry, and static contact angle measurements on unpatterned samples, which were plasma treated under identical plasma conditions. Directed cell attachment studies have also been performed. NIH 3T3 fibroblast cells were used to test the cell adhesion and viability on the various patterned surfaces. PS coated with FC is biologically inert: cells do not adhere on this surface. In contrast, cells proliferate well on surfaces functionalized with organic monomers. Other cell culture experiments and biomolecule patterning will be discussed. @FootnoteText@@footnote 1@ N.A. Bullet, R.D.Short et al. Surface and Interface Analysis. 2001, 31, 1074-1076.

9:20am **BI+PS-FrM4 Combining Pulsed RF Plasma Polymer Coatings with Avidin-Biotin Chemistry for On-Probe Affinity Capture Mass Spectrometry**, ***G.R. Kinsel,*** *M. Li, R.B. Timmons,* University of Texas at Arlington

Matrix assisted laser desorption / ionization mass spectrometry (MALDI-MS) has become a powerful analytical tool for the characterization of proteins. As the effectiveness of the MALDI method has advanced, the need for high-speed isolation and purification of targeted proteins in complex mixtures (e.g. culture media, serum or urine) has increased. The approach described in this presentation focuses on the use of RF plasma polymer coated MALDI probes as platforms for introduction of avidin/biotin chemical modifications. Pulsed RF plasma deposition of allyl amine or vinyl carboxylic acid directly on the MALDI probe surface is used to produce amine modified and carboxylic acid modified surfaces, respectively. Control of the functional group density is achieved through changes in the duty cycle of the pulsed RF plasma. Both amine and carboxylic acid functionalized plasma polymer modified probe surfaces have been investigated as platforms for attachment of avidin or biotin. Testing of the surfaces for peptide/protein isolation based on the targeted properties is performed using various laboratory prepared control mixtures and mixtures obtained from biological sources. In all cases selective capture of the targeted protein/peptide was evaluated through the acquisition MALDI mass spectra using a Bruker BiFLEX linear MALDI TOFMS or a laboratory-constructed linear MALDI TOFMS. Data has been obtained from both avidin and biotin surfaces demonstrating the efficacy of these modified MALDI probe surfaces for achieving on-probe bioselective isolation of target compounds.

9:40am **BI+PS-FrM5 Chemical Modifications of PVC Endotracheal Tubes by RF-Oxygen Glow Discharge Pre-functionalization and NaOH/AgNO@sub 3@ Wet Treatments to Reduce Bacterial Adhesion**, ***D.J. Balazs,*** *K. Triandafillu,* Swiss Federal Inst. of Tech., Switzerland*; P. Wood,* Univ. Hospital of Geneva, Switzerland*; Y. Chevolot,* Goemar Laboratories, France*; C. van Delden,* Univ. Hospital of Geneva, Switzerland*; H. Harms, C. Hollenstein, H.J. Mathieu,* Swiss Federal Inst. of Tech., Switzerland

The use of silver as an antibacterial agent can be traced back to ancient times, and is currently used in several medical applications.@footnote 1@ Bacterial colonization of intubation tubes is responsible for 90% of all nosocomial pneumonia cases, 40 % of which lead to death, despite aggressive antibiotic therapy.@footnote 2@ We have developed an approach based on the surface modification of medical grade poly(vinyl chloride) (PVC) to create an anti-colonization surface, rich in silver ions. The modification consists of an oxygen plasma treatment, followed by a two step wet treatment in sodium hydroxide (NaOH) and silver nitrate (AgNO@sub 3@) solutions. XPS analysis and contact angle measurements were used to investigate the chemical nature and surface wettability of the films following each step of the modification. Saponification with NaOH of esters, like those of PVC plasticizers was determined to be a simple, irreversible method of hydrolysis, producing sodium carboxylate and phthalate salts. Following a subsequent in incubation in the AgNO@sub 3@ solution, XPS showed evidence of a replacement reaction that produced a surface rich in silver ions. The potential of wet treatments that incorporate silver as a germicidal agent was demonstrated in bacterial and biofilm studies, using various P. aeruginosa strains. The native and O@sub 2@ pre-functionalized PVC surfaces submitted to the wet treatments exhibited a 100% reduction in initial bacterial adhesion. The efficacy of the wet treatment to reduce colonization over a longer period was demonstrated as 7-logarithmic drop in biofilm population at 24h and an 8-logarithmic reduction at 72 h, as compared to native PVC substrates. @FootnoteText@@footnote 1@ R.O. Darouiche, (1999) Clin. Infect. Dis. 29, 1371-1377. @footnote 2@ J.L. Vincent, D.J. Bihari, et al., (1995) JAMA 274: 639-644. .

10:00am **BI+PS-FrM6 PECVD Growth and Ion Beam Modification of Polymer Films with Patterned Surface Charge Properties**, ***A. Valsesia,*** *M. Manso, G. Ceccone, D. Gilliland, F. Rossi,* Joint Research Centre, Ispra, Italy

The performance of polymer films in biomedical devices such as DNA arrays and other biosensors depends greatly on the ability to control their surface properties. In fact, surface features determine the ability of the polymer to immobilize a target biomolecule or to give this molecule an orientation towards adsorption. Plasma enhanced chemical vapor deposition (PECVD) of Polymers is an attractive way to produce this kind of films due to the high rate of functional groups obtained at energies ensuring film stability. The density of functional groups can be modified by an Ion Beam Modification. If this last treatment is performed through a mask, the surface remains with regions expressing different responses to chemical groups and environmental free charges (i.e. ions in solution). In this work we have studied the properties of two polymers with contrasted surface charge behavior. Allylamine (AlA) and Acrylic Acid (AcA) films were studied in parallel by Fourier transformed infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) outlining the changes occurred during plasma polymerization and ion beam modification. These spectroscopic results were correlated with wetting and surface charge behavior by performing contact angle and Z-potential measurements. Their stability and ability for protein adsorption was evaluated by using a quartz crystal microbalance (QCM-D). The possible interference with topographic features has been tested by observing the films in an atomic force

microscope, which was further used to monitor electric fields in buffer solutions. From these results we conclude that the combination of PECVD and ion beam modification is an effective way for the growth of polymer films with controlled properties for bio-sensing applications.

10:20am **BI+PS-FrM7 Growth of Biodegradable Thin Films by Methods of Pulsed Laser Deposition**, ***J.M. Fitz-Gerald***, *A.L. Mercado, L. Zhigilei, R. Johnson, C.L. Fraser,* University of Virginia*; J.D. Talton,* Nanotherapeutics, Inc.

Poly(DL-lactide-co-glycolide) (PLGA) is a biodegradable polymer with application in many areas of biomedical field ranging from contact lenses to sustained drug release formulation. In this research thin films (25 nm - 5 microns) of PLGA were deposited onto Si and NaCl wafers, in addition, a specific class of particulate materials (inhaled steroids) ranging from 1-5 microns in size were coated for in-vitro testing. All coatings were processed by both conventional pulsed laser deposition (PLD) and matrix-assisted pulsed laser evaporation (MAPLE) techniques. Film morphology, chemical structure, and decomposition effects were characterized by scanning electron microscopy (SEM), Fourier transform infrared infrared spectroscopy (FTIR), gel permeation chromatography (GPC), nuclear magnetic resonance (NMR), and in-vitro dissolution.

10:40am **BI+PS-FrM8 Study of RGD Peptide and Fibronectin Adsorption on Polymer Surfaces Micropatterned by Cold Plasma and Ion Beams**, ***C. Satriano***, University of Catania, Italy*; M. Manso,* Joint Research Centre, Ispra, Italy*; N. Giamblanco,* University of Catania, Italy*; G. Ceccone, D. Gilliland, F. Rossi,* Joint Research Centre, Ispra, Italy*; G. Marletta,* University of Catania, Italy

Thin films of polycaprolactone (PCL) and polyhydroxymethylsiloxane (PHMS) were patterned by Ar@super +@ ions beams or cold microwave Ar plasmas through Ni masks. The dimensions of the patterns stripes and pitches were typically between 30 and 100 µm. In the case of Ar@super +@ irradiation, the two ion energies of 50 keV and 0.5 keV were used, with fluences ranging from 1x1014 to 5x1015 ions/cm@super 2@. For plasma irradiation, the samples were placed on a grounded or RF biased sample holder (-50V), for different times. The surface structure and composition changes were characterized by spatially resolved X-Ray Photoelectron Spectroscopy (XPS) and Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS). The surface roughness on the micro- and nanometer scale was determined by Atomic Force Microscopy (AFM). The surface charge and dispersive/polar forces distribution were determined by Zeta Potential (ZP) and Surface Free Energy (SFE) measurements respectively. Finally the change of thickness and visco-elastic properties of the films was investigated by the Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) technique. The adsorption of RGD peptide sequences and fibronectin was investigated as a function of the different treatment parameters, including ion energy and dose. The in-situ kinetics of adsorption and modeling of the viscoelastic properties of the adsorbed layers were studied by using QCM-D technique, while the chemical structure and lateral distribution of the adlayers were characterized ex situ by Small Spot XPS, ToF-SIMS Imaging measurements and AFM. The results showed that selective patterning of the adsorbed peptide and fibronectin could be achieved mainly in connection with the polar to dispersive ratio of the surface free energy. In particular, the surface modification seems to affect also the morphology adlayers.

11:00am **BI+PS-FrM9 Chemical Modification of a Three-dimensional Tissue Engineering Polymeric Scaffold by Low-temperature Radio-frequency Plasma Treatment**, ***S. Kumar***, University of South Australia, Australia*; R.St.C. Smart,* University of South Australia*; D.J. Simpson,* University of South Australia and Seoul National University, Korea

The technique of low-temperature radio-frequency plasma has been employed for the chemical modification of Osteofoam, a three-dimensional polymeric (PLGA) tissue engineering scaffold material. The chemical modification in question was aimed at coating Osteofoam with a thin layer of silica, both on its surface as well as in its bulk. For this, Osteofoam cubes of dimensions 12 mm x 12 mm x 12 mm were treated with the plasma generated using tetraethoxysilane (TEOS) as the main precursor. The chemical modification thus achieved was investigated and quantified using the X-ray photoelectron spectroscopy technique, revealing the presence of silica both on the surface as well as in the bulk of Osteofoam samples. The XPS data also suggest that the plasma process developed and employed by us is relativley more efficient at modifying the sample surface than its bulk.

11:20am **BI+PS-FrM10 Deposition of Amine Containing Films from Hyperthermal Silazane and Allyl Amine Ions**, *A. Choukourov, H. Biederman,* Charles University, Czech Republic*; E. Fuoco, S. Tepavcevic,* ***L. Hanley***, University of Illinois at Chicago

Polyatomic ion deposition at ion impact energies below 200 eV is an effective method for the growth of thin organic films on polymer, metal, and semiconductor surfaces. We have previously shown that fluorocarbon and siloxane ions can be employed for the growth and modification of organic thin films on polymer, semiconductor, and metal surfaces.@footnote 1-3@ These films are often similar in chemical composition to plasma polymers, due at least in part the presence of large, hyperthermal positive ions in many plasmas. This work deposits beams of mass-selected 5 - 200 eV silazane and allyl amine ions onto aluminum and silicon substrates. Silazane and allyl amine ions are produced by electron impact ionization of 1,3-divinyltetramethyldisilazane and allyl amine, respectively. These ion-deposited films are analyzed by x-ray photoelectron spectroscopy (XPS) and atomic force microscopy. Chemical functionalization prior to XPS analysis permits the unique identification of primary and secondary amine groups. Secondary amine containing films are shown to form at low silazane ion energies whereas the higher ion energies lead to formation of more inorganic, silico-carbo-nitride-like films. Primary amines are produced by allyl amine ions at various energies. Films grown by allyl amine ion deposition are compared with those produced by plasma polymerization of allyl amine. Effects of film aging in air are also discussed. @FootnoteText@ @footnote 1@L. Hanley and S.B. Sinnott, Surf. Sci. 500, 500 (2002). @footnote 2@P.N. Brookes, S. Fraser, R.D. Short, L. Hanley, E. Fuoco, A. Roberts, and S. Hutton, J. Elec. Spect. Rel. Phenom. 121, 281 (2001).@footnote 3@E.R. Fuoco and L. Hanley, J. Appl. Phys. 92, 37 (2002).

11:40am **BI+PS-FrM11 Plasma Chemistry of Allylamine for the Deposition of Nitrogen-Containing Organic Films**, ***D.C. Guerin***, Naval Research Laboratory, National Research Council*; V.A. Shamamian, R.T. Holm,* Naval Research Laboratory

We studied the chemistry of an allylamine/argon plasma for the deposition of nitrogen-containing organic films. We used in situ mass spectrometry to determine the identity of the molecular ion flux to the deposition surface. Our investigation showed that under the span of powers interrogated (30-100 W) the identity of the ion flux did not substantially change. The total ion current to the deposition surface increased linearly with plasma power. However, the molecular ion mass distribution changed with the plasma pressure. In lower-pressure plasmas, the predominant ions were generated by electron-impact ionization reactions. At higher pressures ions generated by ion-molecule reactions dominate the flux to the surface. We used appearance potential mass spectrometry to confirm the creation of NH@sub 3@ as a by-product of the ion-molecule reactions. The resulting films were characterized optically. The deposition rates were highly dependent on the plasma power. However, the indicies of refraction were similar for the conditions studied. Infrared spectroscopy of the films showed that different plasma conditions resulted in only small changes in film structure. We determined that the film deposition mechanism was not controlled by plasma-ion chemistry. This contrasted with earlier results involving a saturated monomer. However, the film structure was highly dependent on the film thickness. The N-H signal increased greatly in the thicker films. Fluorescamine tagging of the amine groups in the films showed that the primary amine concentration was not well correlated to the intensity of the N-H stretch in the infrared spectra.

Nanometer Structures
Room 317 - Session NS+BI-FrM
Nanotechnology and Biology

Moderator: R.J. Hamers, University of Wisconsin-Madison

9:40am **NS+BI-FrM5 Interaction of Fluorescent Molecules with Metallic Nanoparticles Mediated by Biospecific Interactions**, ***V.H. Perez-Luna***, *K. Aslan, I. Severcan,* Illinois Institute of Technology

Metallic nanoparticles affect the emission characteristics of fluorophores located in their proximity. Here we exploit this strong influence in a system where gold nanoparticles are functionalized with biotin. Preparation of biotinylated gold nanoparticles is performed in the present of a nonionic surfactant to ensure their stability. The interaction of these biotinylated gold nanoparticles with Alexa488-labeled anti-biotin in solution was studied by optical absorption spectroscopy and fluorescence spectroscopy. It was found that reduction or enhancement of fluorescence emission

could result when Alexa488-labeled anti-biotin interacted with biotinylated gold nanoparticles. This depended on the surface coverage of biotin groups, the concentration of antibody and the concentration of biotinylated gold nanoparticles. Introduction of soluble biotin to dissociate the bound antibodies from the surface of the nanoparticles reversed the signals observed previously. These observations can be explained in terms of the competing effects that metallic nanoparticles can have on emission of fluorescence. Quenching of fluorescence can occur when the fluorophores are in close proximity to the metallic surfaces. However, metallic nanoparticles can also enhance the excitation intensity due to concentration of the incident field in the vicinity of the nanoparticles. Additionally, metallic surfaces can also provide additional pathways for radiative decay of the fluorophores. These concepts will have important implications for novel materials in fluorescence detection.

10:00am **NS+BI-FrM6 Real-Time, Label Free Biosensing using Immobilized Gold Nanoparticles: Influence of Nanoparticle Size on Sensor Performance**, ***N. Nath***, *A. Chilkoti,* Duke University

We recently demonstrated a label-free optical sensor to quantify biomolecular interactions in real-time that exploits the surface plasmon resonance effect exhibited by noble metal nanoparticles (nanoSPR). The sensor monitors changes in the extinction spectrum of a monolayer of gold nanoparticles on glass as a function of biomolecular binding. We have previously shown that 13 nm diameter gold nanoparticles can monitor the binding of streptavidin to biotin with a detection limit of 16nM. The performance of the biosensor is controlled by the size, shape and dielectric constant of the metal nanostuctures, and their interparticle spacing. As a first towards optimization of the nanoSPR sensor, we investigated the size of gold nanoparticles on sensor performance. Monodisperse gold nanoparticles were chemically synthesized with diameters ranging from 12 nm to 50 nm. The extinction spectrum of the monolayers of gold nanoparticles of all sizes exhibited both a red shift as well as an increase in the extinction at peak wavelength as a function of bulk solution refractive index. However, sensitivity, defined as change in extinction per unit change in bulk refractive index, increases with an increase in particle size and reaches a maximum value of 1.42 for a particle size of 39 nm. Second, the sensing volume of the immobilized gold nanoparticles, defined as the distance from the surface within which a bulk refractive index change will result in a change in the optical signal, increases with particle size and peaks for 39 nm diameter nanoparticles. Based on these results, an optimized sensor was fabricated using 39 nm gold nanoparticles, and its detection limit for biotin-streptavidin binding was found to be ~1 nM. NanoSPR on a chip is attractive for biosensing because of simple solution based assembly and ability to measure extinction spectrum using widely available UV-vis spectrophotometers.

10:20am **NS+BI-FrM7 Ultrasensitive Nanowire Sensor for Drug Discovery and Medical Diagnostics**, ***W. Wang***, Harvard University

Semiconductor nanowires represent a novel class of nanostructured materials with a wide range of future applications from molecular electronics to biotechnology. Using appropriate fabrication procedures, our group has previously demonstrated that field-effect transistors (FETs) made from p-type Si nanowires possess electronic characteristics exceeding that of conventional planar devices. This outstanding electronic property makes nanowire FETs ideal transducers in a sensor system with label-free, real-time detection capability. Furthermore, sensors made from Si nanowires offer additional advantages over other type of sensors including the ease to differentially modify many nanowires for multiplexed sensing, the potential to be very small and inexpensive, and most importantly the unparalleled extreme sensitivity to the point where single molecule detection is possible. With successful chemical modification to covalently immobilize biological receptors onto the surface of nanowires, we showed that a nanowire FET can be configured into a nano-scale sensor and the binding of charged ligands to the receptors generates specific electrical responses in a quantitative manner. We first applied this strategy to develop a sensitive detector for prostate cancer by measuring the levels of PSA, a marker for prostate cancer. The sensor was shown to detect PSA as low as 0.025 pg/ml (7fM). In addition to medical diagnostics, the combined advantages of label-free detection and extreme sensitivity offer a unique opportunity to configure the nanowire sensors into a drug discovery platform. Using Abl kinase/ATP/Gleevec as a model pathological system (in chronic myeloid leukemia), we have demonstrated the possibility to visualize drug action, or small molecule/protein interactions in real time. Lastly, because of the high sensitivity inherent to the nanowire sensors, individual binding/unbinding events of single molecules can be resolved electrically.

10:40am **NS+BI-FrM8 Nanopores in Ultrathin MOS-compatible Membranes for Electrical Detection of DNA**, ***T. Kim***, *J. Heng, V. Dimitrov, C. Ho,* University of Illinois at Urbana-Champaign; *A. Kornblit, F. Klemens, J. Miner, W. Mansfield, C. Pai, T. Sorsch,* New Jersey Nanotechnology Consortium; *G. Timp,* University of Illinois at Urbana-Champaign

We are developing a revolutionary type of silicon integrated circuit that incorporates MOS technology with an on-chip nano-pore mechanism for directly sensing the electrical activity of bio-molecules such as ions, proteins or DNA. The electronic detection of biological analytes could have several advantages over the conventional scheme, fluorescent microscopy, which is used so prevalently in biology to discriminate the experimental outcomes. For example, if each analyte has a characteristic signature, then an electronic biosensor could facilitate the analysis of the data by eliminating the need for sensitive dyes, thereby improving the dynamic range for detection. We have recently discovered a method to produce ~1-2nm diameter pores (a size comparable to the secondary structure of a protein) in membranes made from materials such as Si, SiO@sub 2@, and Si@sub 3@N@sub4@ that are compatible with MOS fabrication technology. We have adopted this method to create nano-pores spanning a high quality ~2-5nm thick SiO@sub 2@ membrane that constitutes part of the gate electrode in a Metal-Oxide-Semiconductor Field Effect Transistor (MOSFET) amplifier. Here, Hwe report on the fabrication of nanometer-scale pores in MOS compatible materials using a high voltage, tightly focused electron beam, and on time-resolved measurements of the transport of 100bp to 1500bp DNA through a range of pore diameters (2-8nm) and membrane thicknesses (2-30nm).

11:00am **NS+BI-FrM9 Electrically Switchable Nanostructured Superhydrophobic Surfaces**, ***J.A. Taylor***, New Jersey Nanotechnology Consortium; *T.M. Schneider, S. Yang,* Bell Laboratories, Lucent Technologies; *A. Kornblit,* New Jersey Nanotechnology Consortium; *T.N. Krupenkin,* Bell Laboratories, Lucent Technologies

Dynamically switchable nanostructured surfaces are investigated. Behavior of liquids on these surfaces is studied both experimentally and theoretically. Three major states of a liquid drop on these surfaces are demonstrated. The states include highly mobile rolling ball, immobile droplet, and complete wetting state. The transitions between these states were dynamically induced by applying a voltage between a liquid and a nanostructured substrate. Droplet contact angle was measured as a function of applied voltage and nanostructured layer geometry. The obtained results show quadratic dependence of the cosine of the contact angle on voltage, which is in good agreement with the typical electrowetting behavior. The details of interaction of liquids with the nanostructured layer were investigated using SEM technique. The proposed approach potentially allows novel methods of manipulating microscopically small volumes of liquids. This includes essentially frictionless liquid transport, the ability to selectively immobilize the droplets at any given time or position, as well as dynamic control over the penetration on liquids through the nanostructured layer. The obtained results potentially open new and exciting opportunities in microfluidics, chemical microreactors, bio/chemical detection, thermal management of microelectronics, bio-optics, and many other areas.

11:20am **NS+BI-FrM10 Engineering Information Processing in Biological Systems**, ***R.H. Blick***, University of Wisconsin-Madison **INVITED**

The key aspect of this work is to present methods for understanding and engineeringinformation processing in nanoscale biological systems. The systems we are focusing on are nanometer-sized ion channels integrated in high frequency circuits. The ion channels are embedded in bilipid membranes, which are brought to microstructured glass chips for direct transport measurements. Recording the passage of ions is successfully performed and first results on high-frequency response are shown.

Author Index

Bold page numbers indicate presenter

Author Index

Author Index