Biomaterial Interfaces Group
Room 613/614 - Session BI-MoM

Biosensor-Biology Interface

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

8:20am **BI-MoM1 TOF-SIMS Investigation of the Immobilization Process of PNA and DNA Biosensor Chips**, ***H.F. Arlinghaus***, *C. Höppener, J. Drexler, M. Ostrop,* Physikalisches Institut der Universität Münster, Germany

A novel DNA sequencing method is described that uses peptide nucleic acid (PNA) hybridization biosensor chips. PNA is a synthesized DNA analog, in which both the phosphate and the deoxyribose of the DNA backbone are replaced by polyamides. This DNA analog retains the ability to hybridize with complementary DNA or RNA sequences. Because the backbone of DNA contains phosphates, of which PNA is free, an analysis technique that identifies the presence of phosphates in a molecular surface layer allows the use of unlabeled DNA for hybridization on a biosensor chip. We used TOF-SIMS to investigate its ability to distinguished between PNA and DNA molecules on surfaces, as well as the PNA and DNA immobilization process. For this purpose we immobilized silane SA-layers on UV/ozone treated silicon wafers and bonded PNA and DNA with different concentrations to these layers. It was found that the immobilization process is strongly dependent on the concentration and the immobilization time and that under optimized conditions, PNA and DNA can be covalently bonded to the silane SA-layers. A comparison between positive and negative TOF-SIMS spectra showed that the masses corresponding to PO@sub 2@@super -@, PO@sub 3@@super -@ and H@sub 2@PO@sub 4@@super -@ provide the best correlation to DNA presence. The phosphate yield could be significantly increased with polyatomic ion bombardment. Temperature-programmed SIMS (TP-SIMS) was used to measure the thermal stability of the immobilized layers showing that characteristic silane fragment ions decrease at a temperature of about 70°C. It can be concluded that the combination of TOF-SIMS and TP-SIMS provides a very useful technique for examining the complexity of the immobilization and hybridization processes of nucleic acid and that TOF-SIMS has the potential for providing a rapid method for DNA/RNA sequencing and diagnostics.

8:40am **BI-MoM2 Characterization and Quantitation of DNA on Gold**, ***A.B. Steel***, Gene Logic, Inc.*; R.L. Levicky,* Columbia University*; T.M. Herne, M.J. Tarlov,* National Institute of Standards and Technology

The interaction of DNA with gold has been characterized using a number of analytical techniques. The role of structural aspects of oligonucleotides, 8 to 48 nucleotide strands, on immobilization on gold has been investigated using electrochemistry, phosphorimaging, FT-IR spectroscopy, and neutron reflectivity. The value of incorporating a substrate-specific binding group was confirmed. In the case of binding to gold, the substrate-specific group is a thiol (SH). The packing density was studied as a function of the oligonucleotide length. The packing density is roughly constant for oligonucleotide segments less than 16 nucleotides in length. Longer strands pack on the surface at a density that is inversely proportional to the number of nucleotides in the segment. The data suggests that the conformation of single-stranded DNA transitions from a 'rigid rod' to a 'flexible coil' near this 16 nucleotide segment length.

9:00am **BI-MoM3 Gene Engineering for Biosensor-Biology Interface**, ***M. Aizawa***, *E. Kobatake, Y. Yanagida, T. Haruyama,* Tokyo Institute of Technology, Japan **INVITED**

Biosensor technology has made a remarkable progress in these three decades, which may be characterized by unique integration of immobilization and measurement technologies in the first generation, effective employment of advanced technologies such as microelectronics in the second generation and its own development based on generic technology in the third generation. Up to the second generation, the progress of biosensor technology has been realized by adopting the related technologies. It should be regretful, however, that no biosensor material, for instance, has been designed or synthesized for its own purpose. Much effort has been devoted to adopting a native biomolecule to fit a biosensor. It is not until the third generation that biosensor material is designed for its own purpose on the basis of advanced technology. For the progress of biosensor technology, sensing material design technology should be advanced in harmonization with process technology as well as system technology. As far as sensing material design technology, we should concentrate on designing biosensing materials on the basis of gene engineering for the development of the third generation of biosensors. Gene engineering may fall in three categories of engineering including gene designing of proteins, designing of gene expression process and gene diagnosis. In this paper, both gene designing of proteins and designing of gene expression processes for biosensing are described with focusing on our current achievement.

9:40am **BI-MoM5 Cell-Transistor Coupling**, ***A. Offenhäusser***, Max-Planck Institute for Polymer Research, Germany

In recent years it became conceptually feasible to study small networks of synaptically interactive neurons in vitro. Input (stimulation of a single neuron) and output (recording of electrical signals from individual neurons) control in such a neural network could be achieved by direct coupling of the neural electrical signals to a field-effect transistor (FET) device and metal microelectrodes, opening up the possibility for two-way, nontoxic communication between chips and nerve cells. However, the cell-device coupling is not very well understood and the control of this coupling challenging. An extracellular recording system has been designed for the detection of electrical cell signals.@footnote 1@ A field-effect transistor (FET)@footnote 2@ array has been fabricated which consists of p-channel or n-channel FETs with non-metallized gates. The size of the gates of the 16 FETs are from 16x3 um2 down to 5x1 um2 and are arranged in a 4x4 matrix on 200 and 100 um centers. On the other side extended gate electrode (EGE) arrays were used which are arranged in a 8x8 matrix on 200 and 100 um centers. The gate electrodes are made from gold, titanium and silicides with diameters down to 6 um. The cell-device coupling has been studied using various cell types e.g. neuronal cells, cardiac myocytes, and cells from cell lines. The recorded signals will be discussed on the base of a point contact model where contributions from passive as well as active membrane properties are included. @FootnoteText@ @footnote 1@ C. Sprössler, D. Richter, M. Denyer, A. Offenhäusser, Biosens.& Bioelec. 13, 613-618 (1998). @footnote 2@ A. Offenhäusser, C. Sprössler, M. Matsuzawa, W. Knoll, Biosens.&Bioelec. 12, 819-826 (1997).

10:00am **BI-MoM6 Specific Interactions between Bitoin and Avidin Studied by AFM using the Poisson Statistical Analysis Method**, ***T.P. Beebe***, *Y.-S. Lo, N.D. Huefner, W.S. Chan, B.A. Shiley, F. Stevens,* University of Utah

The interactions between biotin and avidin or streptavidin, a prototypical example of specific biological ligand-receptor systems, were studied by atomic force microscopy (AFM). A unique statistical analysis method which makes use of the properties of the Poisson distribution was applied, and the rupture strength of an individual interaction was obtained from the total pull-off forces measured by the AFM. Tip- and surface-modification chemistries were investigated by X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (TOF-SIMS). The Poisson analysis method has several advantages. It requires no assumptions about the surface energies or contact area between the AFM tip and the substrate, it is not limited by the force resolution of the instrument, and the number of measurements required to extract the individual unbinding force is significantly lower than that required by some other methods. It has been shown that bond rupture strengths are dependent on the rate and duration of force loading applied during the unbinding process. The dynamic nature of bond strengths under external forces has been explored in theory and by several computer simulations, however, only very few experimental studies have been reported. In the present study, we measure the unbinding force of the biotin-streptavidin pairs under various loading rates. The loading rate dependence of bond rupture forces and the comparison of our experimental data to the known thermodynamic properties of the system will be discussed.

10:20am **BI-MoM7 Manipulation of Cellular Interactions with Biomaterials toward aTherapeutic Outcome**, ***M.D. Pierschbacher***, Integra LifeSciences Corporation **INVITED**

A new way of manipulating the manner in which cells interact with biomaterials was made possible with the discovery of arginine-glycine-aspartic acid (RGD) as a major cell recognition signal in the extracellular matrix. This RGD signal has been incorporated into synthetic compounds that can function as antagonist or agonist for a class of cell surface receptors called integrins. In the agonist mode, these compounds can be coupled or bound to wide variety of biomaterials to present a target for the physiological interaction of cells with the surface of these materials through one or more of the integrin types expressed on the cell surface. The agonist activity of these compounds is evident from an in vivo response of faster and more complete tissue integration and a reduction in foreign body response. There are more than 25 different integrin subtypes, and different cell types express a unique subset of these on their surface. More than half of the integrins recognize and bind to a form of the RGD

signal in their natural ligand and can distinguish one form of RGD from another. Specific tissue responses such as vascualization or mineralization can be achieved by immobilizing compounds with the appropriate integrin specificity.

11:00am **BI-MoM9 Investigations into Peptide-tethered Lipid Bilayer Membranes***, **L.C.R. Naumann,*** Max-Planck Institute for Polymer Research, Germany*; E.K. Schmidt,* Institute of Physical and Chemical Research (RIKEN), Japan*; A. Offenhäusser, W. Knoll,* Max-Planck Institute for Polymer Research, Germany

Lipid-functionalised thiopeptides were employed to form peptide-tethered lipid monolayers on gold substrates. Upon fusion of liposomes these monolayers formed tethered lipid bilayer membranes (tBLM's) well designed to incorporate membrane proteins such as H+-ATPase, Na,K-ATPase, cytochrome c oxidase (COX) and the acetylcholine receptor (AChR). Pure peptide-tBLMs and those mixed with a lateral spacer such as thioethanol were investigated with regard to protein incorporation which was followed by surface plasmon resonance spectroscopy (SPS). Electrical properties were assessed simultaneously by impedance spectroscopy (IS). Fluorescence microscopy showed the bilayers to be homogeneous, however, by FRAP measurements fluidity of the membranes was not observed. Fluorescence microscopy was also used to determine the surface concentration of fluorescein labeled COX. Binding assays were performed by SPS of agonists and antagonists of the receptors such as cyochrome c and bungarotoxin. The specificity and sensitivity of the binding assays was increased for primary monoclonal and secondary polyclonal antibodies against COX and the AChR by using an extension of SPS, surface plasmon enhanced fluorescence spectroscopy (SPFS). Proton transport through H+-ATPase from chloroplasts was then investigated with either coupled or non-coupled proton discharge at the gold electrode, depending on the applied potential. In cases where faradaic processes were involved, electrochemical techniques were applied, such as square wave voltammetry and chronoamperometry, where as proton transport across the lipid film was followed by IS. Impedance spectra thus showed characteristic changes as a function of adenosin-triphosphate (ATP) and inhibitor (venturicidin) concentration and/or bias potentials.

11:20am **BI-MoM10 Detection of Immobilized Superparamagnetic Nanosphere Assay Labels using Giant Magnetoresistive Sensors***, **M.C. Tondra,*** Nonvolatile Electronics*; M. Porter,* Iowa State University

Commercially available superparamagnetic nanospheres are commonly used in a wide range of biological applications, particularly in magnetically assisted separations. A new and potentially significant technology involves the use of these particles as labels in nanomagnetic assay applications. This labeling is analogous to that of flourescent beads: the beads are excited and detected with magnetic fields rather than with photons. A major advantage of this technique is that the means for label excitation and detection are easily integrable on a silicon circuit. A preliminary study of this technique demonstrated its basic feasibility, and projected a sensitivity of better than 10@super -12@ Mole.@footnote 1@ This paper presents detailed magnetic and geometric design considerations for this type of assay, and addresses the range of applications over which the technique is appropriate. It is shown that, with proper sensor design and immobilization techniques, integrated magnetoresistive sensors can be used to easily detect the presence or absence of single 1000 nm magnetic microspheres immobilized on the surface of a giant magnetoresistive sensor. Detection of microsphere labels in the 10 to 100 nm range may also be possible if other sensing parameters are compatible. @FootnoteText@ @Footnote 1@ David R. Baselt, Gil U. Lee, Mohan Natesan, Steven W. Metzger, Paul E. Sheehan, and Richard J. Colton, "A Biosensor Based on Magnetoresistance Technology," Biosensors and Bioelectronics, Vol. 13, pp. 731-739 (1998).

11:40am **BI-MoM11 Characterization of S-layer-supported Bilayer Lipid Membranes***, **B. Schuster,*** *D. Pum, U.B. Sleytr,* Universität für Bodenkultur Wien, Austria

Biosensors, based on electrical detection of specific ligand binding become of increasing importance over the last years. Reliable application make great demands on these designed systems like stable membranes with sufficient fluidity and controlled, orientated linkage of sensing molecules to benefit from the various biological interactions. One promising strategy is the application of bacterial-cell-surface-layers (S-layers) as biocompatible and supporting structures for bilayer lipid membranes (BLM's). S-layer are the simplest self-assembly systems that produce crystalline, mono-molecular, isoporous protein lattices with well-defined topographical and physico-chemical properties. Recent studies on S-layer-supported BLM's demonstrated, that the fluidity of the BLM is retained and an enhanced stability is observed as these BLM's reveal a decreased tendency to rupture in the presence of ionophores or pore-forming proteins. Furthermore, S-layer proteins can be recrystallized on solid supports like gold or silicon wafers, and provide a biocompatible, water-containing layer. Attached BLM's exhibit an increased fluidity compared to dextran- or silane-supported BLM's and the stability is significantly enhanced. Thus, S-layer can be used as an alternative to soft polymer cushions and to common tethers to support functional BLM's. Additionally, a second S-layer can be recrystallized on the opposite face of the membrane. This will allow to employ the intrinsic molecular sieving properties and to immobilize a range of biologically functional molecules in a well-defined position and orientation on the S-layer lattice. Thus, BLM's with attached S-layer(s) in combination with new sensor technology might play an important role in the development of biosensors.

Biomaterial Interfaces Group
Room 613/614 - Session BI-MoA

Protein Solid-Surface Interactions I

Moderator: G.U. Lee, Naval Research Laboratory

2:00pm **BI-MoA1 Formation of 2D Crystals of Proteins on Solid-Supported Lipid Bilayers Followed by AFM**, ***A. Brisson,*** University of Groningen, The Netherlands, Netherlands; *I. Reviakine, W. Bergsma-Schutter, N. Govorukhina, S. Mazeres,* University of Groningen, The Netherlands

The possibility of assembling macromolecules or particles in a controlled manner on solid surfaces constitutes a central issue in the emerging field of nanotechnology. Methods for ordering proteins as 2D crystals or helical arrays at the level of lipid surfaces have received particular attention in molecular structure determination by electron crystallography. One of these methods, called the lipid layer crystallization method, uses the specific interaction between a protein and a ligand coupled to a l ipid molecule incorporated in a lipid monolayer at the air-water interface.@footnote 1@,@footnote2@,@footnote 3@ Our objective has been twofold: 1) to extend this strategy to the case of solid-liquid interfaces; 2) to develop an in situ method for following the formation of prot ein 2D crystals. Using Atomic Force Microscopy, both processes of formation of lipid bilayers on solid supports (SPB), and of protein 2D crystallization on SPB could be revealed in real time, in a natural aqueous environment, at sub-molecular resolution.@footnote 4@,@footnote 5@ Results will be presented on the crystallization of annexin V, an inhibitor of blood coagulation which binds specifically to negatively charged lipids, and will be compared to Electron Microscopy data of 2D crystals formed on lipid monolayers. This novel experimental approach offers exciting opportunities in basic science for investigating crystallization processes and provides an adequate technology for fabricating protein-containing biofunctional surfaces. @FootnoteText@ @footnote 1@Uzgiris, E.E. Kornberg, R.D., Nature 301 (1983) 125. @footnote 2@Brisson A. et al., J. Crystal Growth 196 (1999) 456. @footnote 3@Brisson, A. et al., in Crystallization of Proteins and Nucleic Acids, A Practical Approach, Oxford Univ. Pres (in press). @footnote 4@Reviakine, I., Bergsma-Schutter, W. Brisson, A., J. Struct. Biol. 121 (1998) 356. @footnote 5@Reviakine, I. Brisson, A., (submitted). .

2:20pm **BI-MoA2 Light Activated Affinity Micropatterning of Proteins**, ***A. Chilkoti,*** *Z.-P. Yang, W. Frey,* Duke University; *T. Oliver,* Food and Drug Administration

Biomolecular patterning has diverse applications, which range from modulation of cell-substrate interactions in biomaterials and tissue engineering, to the fabrication of multianalyte biosensors, clinical assays, and genomic arrays. Motivated by these applications, we have developed a method to micropattern proteins on well-defined gold substrates, which we term light-activated affinity micropatterning of proteins (LAMP). LAMP is a multi-step patterning process: first, a gold substrate is functionalized with a binary mixture of 11-mercaptoundecanol and 16-mercaptohexadecanoic acid to provide a non-fouling, reactive self-assembled monolayer (SAM) template on gold. Next, the carboxylic acid end groups in the binary SAM are coupled to methyl @alpha@-nitropiperonyloxycarbonyl biotin succinimidyl ester (caged biotin ester) through a diamine linker. Deprotection of caged biotin by spatially-defined uv illumination at 350-360 nm reconstitutes biotin in the illuminated region, and subsequent incubation with streptavidin results in selective binding of streptavidin to regions that were previously deprotected. We have investigated and optimized LAMP by contact angle goniometry, ellipsometry, surface plasmon resonance, and X-ray photoelectron spectroscopy to maximize ligand density and pattern contrast. Micropatterning of streptavidin and an anti-biotin monoclonal antibody has been demonstrated with a spatial resolution of ~5 microns by imaging ellipsometry and confocal light microscopy of fluorophore-derivatized proteins. LAMP can be further extended to allow spatially-resolved micropatterning of multiple biomolecules by repeated cycles of spatially-defined deprotection, streptavidin incubation, followed by binding of the biotinylated moiety of interest.

2:40pm **BI-MoA3 Oriented Protein Binding via Silane-SAMs Using His-tagged Proteins**, *C. Hoffmann,* University of Stuttgart, Germany; *H. Brunner,* ***G.E.M. Tovar,*** Fraunhofer Institute for Interfacial Engineering and Biotechnology, Germany

Oriented immobilization of proteins is a promising way of retaining protein activity on artificial surfaces.@footnote 1@ Modern protein engineering has already developed suitable tags for this purpose. Surface engineering is now required to provide complementary surfaces. We present experimental data on surface functionalization of silicon oxide surfaces using silane SAMs and the subsequent oriented binding of his-tagged proteins. We synthesized nitrilo-triacetic acid (NTA) silanes for direct attachment to silicon oxide surfaces, and NTA derivatives@footnote 2,3,4@ reactive to surfaces functionalized using epoxy terminated silanes. The stepwise surface functionalization and the binding capacitiy of the NTA surfaces for histidine peptides and his-tagged proteins has been investigated using XPS, MALDI-TOF-MS, contact angle measurements and in situ-ellipsometry. @FootnoteText@ @footnote 1@ Rao, S.V., Anderson, K.W., Bachas, L.G., Mikrochim. Acta, 1998, 128, 127-143. @footnote 2@ Dietrich, C., Schmitt, L., Tampe, R., Proc. Natl. Acad. Sci. USA, 1995, 92, 9014-9018. @footnote 3@ Sigal, G.B., Bamdad, C., Barberis, A., Strominger, J., Whitesides, G.M., Anal. Chem., 1996, 68, 490-497. @footnote 4@ Liley, M., Keller, T.A., Duschl, C., Vogel, H., Langmuir, 1997, 13, 4190-4192.

3:00pm **BI-MoA4 Complement Activation by IgM Immobilized on Methylated Silicon**, ***P. Tengvall,*** Linkoping University, Sweden; *A. Askendal,* Linkoping University; *K.I. Lundström,* Linkoping University, Sweden

The activation of the complement system by IgM immobilized on methylated silicon was studied by ellipsometry/antibody techniques. The IgM coated surfaces were incubated in normal human- and depleated sera at 37Â°C up to 90 minutes, and the deposited amounts of characteristic proteins probed by antibodies. The results indicate, surprisingly, that the so prepared model surface activates the human complement in a manner independent of calcium, although via the classical pathway.

3:20pm **BI-MoA5 Theoretical and Experimental Studies of the Interaction of Water with Oligo (ethylene glycol) Terminated Self Assembled Monolayers**, *R. Wang, H.J. Kreuzer,* Dalhousie University, Canada; *A.J. Pertsin,* ***M. Grunze,*** University of Heidelberg, Germany

The interaction of water with poly(ethylene glycol) and oligo(ethylene glycol) moieties in alkanethiolate SAMs is strongly dependent on the molecular conformation, lateral density and external electrical fields. In this contribution we will present ab initio quantum mechanical results and Monte Carlo Simulations on the ethylene glycol / water interaction and correlate them with vibrational sum frequency generation spectroscopic results of the OEG / water interaction,@footnote 1@ force distance measurements between neutral and charged AFM tips and OEG SAMs of different density,@footnote 2@ and single molecule force spectroscopy.@footnote 3@ @FootnoteText@ @footnote 1@M. Zolk, M. Buck, F. Eisert, M. Grunze, in preparation @footnote 2@K. Feldman, G. Habhner, N.D. Spencer, P. Harder, M. Grunze, JACS, submitted @footnote 3@F. Oesterheld and H. Gaub, New Journal of Physics 1 (1999), 6.1-6.11 3.

3:40pm **BI-MoA6 Protein Resistant Surfaces Based on Reactions of Thiol-Terminated Polyethylene Oxides with Gold**, ***J.L. Brash,*** *Y.J. Du,* McMaster University, Canada

Gold is a relatively inert metal but has strong specific interactions with sulfur functions, particularly thiol. Thiols that contain bioinert or bioactive moieties (eg bioactive peptides containing a terminal cysteine) can thus be chemisorbed to gold films to provide correspondingly bioinert or bioactive surfaces. High surface densities of ligands should be possible due to the abundance of thiol binding sites in the surface. Thiolated gold surfaces thus have great potential in the design of biocompatible materials. We have used this approach in the development of a variety of surfaces based on:(1) suppression of non-specific protein adsorption, (2) selective/exclusive adsorption of a target protein from the contacting biological fluid. In the present work, surfaces have been developed consisting of HS-polyethylene oxides (HS-PEO, expected to minimize non-specific protein adsorption) chemisorbed to gold films deposited on polyurethane or silicon. PEOs of varying MW and architecture (linear, star) were reacted with mercaptoacetic acid to attach terminal thiol groups. HS-polypropylene oxides (HS-PPO) were used as controls, not expected to reduce protein adsorption. Chemisorption of the PEOs and PPOs to gold was verified by XPS. The adsorption of fibrinogen and albumin from buffer and plasma was studied by radioiodination methods. It was found that: (a) protein adsorption is low on all the modified surfaces and decreases with increasing molecular weight of the PEO, (b) with MeO as the terminal

group at the free end of PEO, the suppression of protein adsorption is less than when OH is the terminal group, (c) chemisorbed PPO suppresses the adsorption of fibrinogen and albumin but not as effectively as PEO. In ongoing work we are investigating surfaces with heparin attached to the free chain end of chemisorbed PEO.

4:00pm **BI-MoA7 Protein Adsorption on Mixed Polyethylene Glycol/Methyl Terminated Alkane Thiol Self-Assembled Monolayers**, ***T. Kim***, *K.D. Hauch, J.W. Rogers, Jr.,* University of Washington

Polyethylene glycol(PEG) functionality has been used in many settings to resist protein adsorption.@footnote 1@ In this work, PEG and methyl terminated alkane thiols were used to form mixed self-assembled monolayers(SAM) on gold.@footnote 2@ These surfaces were used to study fibrinogen(Fg) adsorption. The molar percent of PEG thiol in the assembly solution was varied from 0-100% to vary the amount of PEG thiol on the surface. The mixed SAM's were examined with XPS. The atomic composition of the surfaces assembled from pure methyl thiol and pure PEG thiol agreed with expected values. An increase in ether bonds was seen as the PEG thiol ratio in the assembly solution was increased indicating an increase of PEG thiol in the mixed monolayer. The adsorption of I-125 labeled human Fg from dilute plasma (2 hr, 37 degrees Celsius) was measured. Fg adsorption on the SAM's ranged from 11-70 ng/cm^2, significantly lower than the control, polytetrafluorinated ethylene. The pure PEG thiol monolayer adsorbed 12 times less Fg than the control and 7 times less than the pure methyl SAM. We have demonstrated that the surface composition of the SAM is influenced by the composition of the assembly solution. Increased PEG thiol in the SAM reduced Fg adsorption with the pure PEG monolayer exhibiting the lowest values. Future work will include further surface characterization of the SAM's, adsorption studies with other proteins, and examination of choline or saccharide terminated thiols. @FootnoteText@ @footnote 1@ Lopez, G.P., et. al. Glow discharge plasma deposition of tetraethylene glycol dimethyl ether for fouling-resistant biomaterial surfaces. J. Biomed. Mater. Res. 1992, 26, 415-439 @footnote 2@ Ulman, A. Formation and Structure of Self-Assembled Monolayers, Chem. Rev. 1996, 96, 1533-1554

4:20pm **BI-MoA8 Properties of Oligo(ethylene glycol)- Terminated Alkanethiolates on Gold: Influence of Lateral Hydrogen Bonding**, ***R. Valiokas***, *S. Svedhem, S. Svensson, M. Östblom,* Linköping University, Sweden*; U. Gelius,* Uppsala University, Sweden*; B. Liedberg,* Linköping University, Sweden

An exhaustive study on properties of a class of oligo(ethylene glycol) (OEG)-terminated alkanethiolates on gold is presented. Contact angle goniometry, ellipsometry, infrared reflection-absorption spectroscopy, and X-ray photoelectron spectroscopy indicate formation of highly ordered self-assembled monolayers (SAMs), and the compounds containing amide moieties display lateral hydrogen bonding. The conformation of the OEG portion is shown to depend on the oligomer chain length and the lateral interactions, as evidenced by a different phase behavior of analogous OEG compounds which contain no amide group, as well as by using mixed SAMs of shorter and longer OEG chains. The assembly kinetics is found to be important in order to fine-tune the dominating phase of the SAMs. Furthermore, the thermal properties of the SAMs are investigated and temperature-dependent phase behavior is used to understand the nature of the conformational transitions of the OEG portion in relation to hydrogen bonding. Thus, the specifically introduced lateral interactions are shown to be not only a useful tool to improve the order and stability of the SAMs, but also to control conformational/functional properties of terminal groups which in this case are oligomers. Possible further modifications of such OEG SAMs and their bio-oriented applications are discussed.

4:40pm **BI-MoA9 1-Thiaoligo(Ethylene Oxide) SAMs: Biomimetic Matrix Structure Tuned via Hydrophilic Spacer Length & Packing Density**, ***T. Petralli-Mallow***, *D.J. Vanderah, C.W. Meuse, A.L. Plant,* National Institute of Standards and Technology

Interest in reconstituting transmembrane proteins into supported cell membrane mimics has led to development of novel tethering molecules. In order to introduce a flexible and fluid hydrophilic region at the proximal side of a supported lipid bilayer, oligo(ethylene oxide) moieties have been used as spacers between the sulfur and the alkane chain of alkanethiols. Our group has previously shown that the ethylene oxide moiety adopts a 7/2 helical conformation in self-assembled monolayers (SAM)s of both 1-thiahexa(ethylene oxide) (HS(EO)@sub 6@) decane and HS(EO)@sub 6@ octadecane on gold, indicating that the alkane chain is not the driving force for the helical conformation. Ethylene oxide structure may be controlled by packing density. For example, infrared analysis of mixed monolayers of HS(EO)@sub 6@-decane and phospholipids transferred from the air/water interface indicate that at low packing densities the EO region is disordered, but at higher packing densities the EO segment can assume the helical structure. For a series of decane-terminated 1-thiaoligo(ethylene oxide) SAMs with varying lengths of ethylene oxide spacers, infrared spectroscopy and sum frequency generation indicate that the conformation of the ethyleneoxide moieties may be controlled by their length. The conformation of the ethylene oxide segment is an extended all-trans chain in HS(EO)@sub 4@ SAM, a 7/2 helix in HS(EO)@sub 5-7@ SAM, and a less ordered conformation in the HS(EO)@sub 8@ SAM. The ability to control order via spacer length and packing suggests that HS(EO)@sub n@ SAMs may be successful platforms for biomimetic materials incorporating transmembrane proteins.

5:00pm **BI-MoA10 Reduction of Protein Adsorption on Polyethylene Glycol Covered Silica Surfaces**, ***N.A. Alcantar***, *T.L. Kuhl,* University of California at Santa Barbara*; E.S. Aydil,* University of California at Santa barbara*; J.N. Israelachvili,* University of California at Santa Barbara, US

Over the last decades, a large fraction of the scientific community has been dedicated to developing synthetic materials that can be used as implants or replacements for bones, organs, joints, tissues, skin, etc. These artificial materials must not only accomplish a specific function, but also be inert in the biological environment to which they are exposed. In general, the ability of a surface to reject proteins is a parameter used for determining its biocompatibility. Surfaces covered with polyethylene glycol (chemically (PEG, OH-(CH@sub 2@- CH@sub 2@-O)@sub n@-H) have been shown to be biocompatible as PEG enhances nonimmunogenecity, nonantigenicity and protein rejection. In order to produce a generic biocompatible surface coating, we have developed a direct method for grafting PEG onto amorphous activated silica surfaces or films. We first deposited an amorphous silica film by plasma enhanced chemical vapor deposition from SiH@sub 4@ and O@sub 2@ gases, which provides the flexibility to coat diverse materials with different shapes. These silica films were then activated by exposure to water plasma, thus increasing the number of hydroxyl groups on the surface. The silanols (Si-OH) on the resulting surface chemically react with the hydroxyl end of the PEG chain forming an ester bond, Si-O-C. The surface reaction was monitored using Attenuated Total Reflection Fourier Transform Infrared spectroscopy. Two representative fluorophore-labeled proteins were used in this study because of its relatively abundance in the blood stream. Measurements of protein absorption by fluorescence microscopy showed that the PEG coated surfaces significantly inhibit protein adsorption.

Biomaterial Interfaces Group
Room 613/614 - Session BI-TuM

Protein Solid-Surface Interactions II
Moderator: J.L. Brash, McMaster University

8:20am **BI-TuM1 Functionality of a Model Protein at Nanostructured Surfaces**, ***D.S. Sutherland***, Chalmers and Gothenburg University, Sweden; *M. Broberg, H. Nygren,* Gothenburg University, Sweden; *B. Kasemo,* Chalmers and Gothenburg University, Sweden

Nanofabricated surfaces can be used to study the influence of surface topographic features on the behaviour of a model protein. Colloidal lithography was used with simple lift-off steps to create surfaces with defined nanotopography. The functionality of equal-quantities of fibrinogen molecules bound at surfaces containing pits of 40nm diameter or 110nm diameter was probed. The kinetics of binding of unactivated platelets to these surfaces from a static albumin-containing buffer was used as a measure of the functionality/conformation of the fibrinogen molecules. Two sets of samples were prepared, one where the surfaces were chemically homogeneous (titanium oxide) and one where the surfaces additionally contained nanodomains of gold coated in a methyl-terminated self-assembled monolayer at the base of the pits. Platelet binding on fibrinogen adsorbed at flat surfaces and surfaces with 110nm diameter pits showed relatively low binding rates which were not significantly different than that found at control surfaces (with no adsorbed fibrinogen). By contrast platelet binding on fibrinogen adsorbed at surfaces containing 40nm diameter pits gave significantly higher binding rates than both other test surfaces and control surfaces. For these samples with 40nm diameter pits similar results were obtained for both surfaces with homogeneous chemistry and for chemically nanodomained surfaces. These results are interpreted to mean that fibrinogen molecules bound at surfaces with 40nm diameter pits have altered conformation or orientation (compared to flat surfaces or surfaces with larger pits) to make available platelet-binding sites. These pits are smaller that the reported characteristic size of fibrinogen molecules (46-55nm length) and it appears that the effect is the result of topography rather than surface chemistry. These model experiments indicate that the conformation/orientation of individual protein molecules can be influenced by like-sized surface features.

8:40am **BI-TuM2 Hierarchical Ordering of Proteins at Interfaces with a Nanoscale Surface Topography**, ***V. Vogel***, *L. Smith, T. Nguyen, J.R. Dennis,* University of Washington

Elucidating mechanisms by which to control the ordering of proteins at interfaces is of fundamental importance in bioengineering and biotechnology. Whereas major progress has been made recently in stabilizing proteins at interfaces in their native states, and in controlling their orientation, much less is known how to promote their spontaneous self-assembly into a structurally well controlled supramolecular architecture. Here we discuss that nanoscale topographic surface features with ridges the size of natural ECM fibrils have a pronounced impact on protein adsorption, and on the spatial alignment of human dermal fibroblasts and cell-deposited collagen fibrils. Furthermore, by elevating cells above the surface such that they deposit collagen through a porous membrane onto the nanoscale ridges without being in physical contact with the surface, the role of the cells has been separated from the role of topography in collagen type VI deposition and fibrillogenesis. Insight into the mechanisms by which synthetic surfaces manipulate the hierarchical organization of ECM fibrils will be crucial in the rational design of the surface topography of biomaterials and of scaffolds for tissue engineering.

9:00am **BI-TuM3 Protein Adsorption on Solid Surfaces : From Static to Dynamic Properties**, ***P. Schaaf***, Institut Charles Sadron, France **INVITED**

Adsorption processes of proteins on solid surfaces have been investigated over many years but, due to their complexity, it is still difficult to predict their behavior. Indeed, proteins are highly structured polyelectrolytes, polyamphiphiles, which, in addition, are often only marginally stable. While interacting with a solid surface, they often change their structure and parallelly increase their anchoring to the surface. The influence of different parameters entering in these adsorption processes, such as the substrate charge, the substrate hydrophobicity and the protein stability, will be discussed. The dynamics of these processes will, in particular, be mentionned. It will appear that the time scales entering in adsorption processes range from tens of hours down to milliseconds. While the characteristic interaction time needed for fibrinogen, a plasma protein, to bind to a silica surface appears to be of the order of 50ms, the exchange ability of a ribonuclease molecule adsorbed on a titanium oxyde surface changes with a characteristic time of the order of 10 hours. Structural changes are observed in the adsorbed layer over similar time scales. Recent results obtained in this field, in particular by Infrared Spectroscopy and by Atomic Force Microscopy, will be presented

9:40am **BI-TuM5 Protein Adsorption Kinetics: Particle Model and Optical Experiment**, *M.A. Brusatori, C. Calonder,* ***P.R. Van Tassel***, Wayne State University

The adsorption of proteins at the liquid-solid interface is a phenomenon of great importance in bioseparations, biocatalysis, and materials biocompatibility. Adsorption is often accompanied by a surface-induced transition in either internal conformation or molecular orientation. Recently, Van Tassel, et al, presented a model in which the adsorption/transition process is modeled as the sequential surface placement of spreading disks. In this talk, we present a derivation of analytical expressions for the adsorption and spreading probabilities, whose use greatly simplyfies the form of the kinetic equations for this model, using the scaled particle theory (SPT). We also present new experimental data of fibrinogen adsorption onto SiTiO2 and dextran-coated SiTiO2 using optical waveguide lightmode spectroscopy (OWLS). We show that the SPT spreading disk model can accurately predict these and other experimental data as well as those from computer simulation.

10:00am **BI-TuM6 Probing Immobilized Protein Peptide Architectures**, ***S.J.B. Tendler***, *M.M. Stevens, W.C. Chan, M.C. Davies, C.J. Roberts, P.M. Williams, S. Allen,* University of Nottingham, U.K.

The ability to control the assembly of molecular architecture at the nanometre scale is an important research goal. Complex molecular assemblies can be designed and constructed to have applications in several bio-analytical fields, for example, as key components in devices such as biosensors and affinity-based chromatographic supports. We have demonstrated the creation of a higher order molecular assembly which consists of a bis-biotinylated peptidic spacer between two streptavidin molecules. This molecular architecture exploits the strong affinity between streptavidin and biotin to promote self-assembly. Surface plasmon resonance has enabled us to monitor the construction of the multilayer in real time. Atomic force microscopy has been utilized to measure adhesion forces between biotinylated bovine serum albumin functionalized probes and the surface at each stage of the multilayer assembly. This facilitated the determination of surface functionality and associated mechanical properties at each of these stages. An increase in the elasticity of the system was observed once the multilayer was created. It is postulated that unraveling of an alpha-helical component in the conformation of the peptide before rupture of the streptavidin-biotin link may contribute to the increase in molecular elasticity of the multilayer. We have also demonstrated through a trifluoroethanol titration monitored by circular dichroism that variations in the solvent can affect the secondary structure of the peptide linker and hence its mechanical properties. These observations have wide implications for protein immobilization in terms of the precise control of distances of active layers, steric surface barriers, underlying surface forces and hence biological functionality.

10:20am **BI-TuM7 New Platform Technology for the Investigation of Initial Interaction of Adsorption and Cross Linking of Strong Adhesives at Solid Surfaces**, ***H. Elwing***, *F. Hook,* Goteborg University, Sweden

The contacting area between an implanted biomaterial and the surrounding tissue is of critical importance for the functional success of the biomaterial. We try to develop tissue "glues" and we get our biomimic inspiration from marine organisms. Several marine animals and plants living at hard rocks, or man made material have developed successful glues or adhesives for contacting the hard surface. There must be at least two conditions fulfilled for strong adhesion of marine organisms to a flat solid surface. Firstly there must be sufficient strength of molecular adhesion at the liquid/solid interface. Secondly it is required that the adhered molecules are cross-linked at the surface and into the tissue of the organism. We have concentrated our effort to understand more about the cross-linking mechanisms. Unfortunately there are few methods available for measuring cross-linking of biopolymers in real time, which have made research difficult. Consequently, as a first step we have developed a methodological combination of surface plasmon resonance (SPR) and Quarts chrystal microbalance (QCM-D) for simplified analysis of adsorption and cross-linking of marine adhesives, such as mussel adhesive proteins and Barnacle cement, adsorbed as monolayers on flat solid surfaces.

10:40am **BI-TuM8 Molecular Orientation Distributions in Submonolayer Films Corresponding to Quasi-Reversible Electron Transfer Behavior**, *S.S. Saavedra,* ***R.T. Robertson****, N.R. Armstrong, S. Mendes,* University of Arizona

The relationship between the molecular orientation distribution and the electron transfer behavior of immobilized redox proteins has been investigated through the use of novel waveguide-based spectroelectrochemical methods. The ability to probe the electron transfer behavior which corresponds to a particular molecular orientation will provide significant insight into the fundamental electron transfer processes that occur in physiological systems. The orientation geometry is obtained using an electroactive integrated optical waveguide (EA-IOW) format coupled with electroactive total internal reflection fluorescence (EA-TIRF). Orientation distributions of horse heart cytochrome c corresponding to quasi-reversible electron transfer have been characterized and will be discussed here.

11:00am **BI-TuM9 Surface Orientation of Peptides with @alpha@-helix and @beta@-sheet Secondary Structures on Fluorocarbon Substrates**, ***L. Gamble****, J.R. Long, P.S. Stayton,* University of Washington*; D.A. Fischer,* National Institute of Standards and Technology*; D.G. Castner,* University of Washington

The orientation of surface bound proteins can have a significant effect of their function. To aid with the interpretation of Near edge X-ray absorption fine structure (NEXAFS) spectra from adsorbed protein films we haved studied short, well-defined peptide "standards." NEXAFS is a surface sensitive technique that has been used to determine the orientation of polymers and self-assembled monolayers. Here NEXAFS is used to determine the surface orientation of short peptides chains designed to adsorb in @alpha@-helical and @beta@-sheet conformations on hydrophobic surfaces. The N K-edge spectra show an orientation dependence of the N1s to @pi@* peak between the 90° and 20° incident x-ray angles for both peptides adsorbed onto highly-ordered poly(tetrafluoroethylene) (PTFE) surfaces. The results indicate that the @beta@-sheet peptide is adsorbed with the peptide backbone "parallel" to the substrate, while the @alpha@-helix adsorbes with the helical axis parallel to the substrate. Spectra of the O K-edge support these results. The lack of orientational dependence seen for these same peptides adsorbed onto a disordered fluoropolymer surface containing different types of fluorocarbon species indicates the degree of substrate order and/or the type of surface functional groups play a key role in determining the degree of ordering in the adsorbed peptides. NEXAFS spectra were also used to distinguish between the secondary structures of the two peptides. Preliminary NEXAFS results from adsorbed protein films show that orientataion of the peptide backbone is only observed for non-gobular proteins such as fibrogen and fibronectin. Gobular proteins such as albumin do not exhibit any preferrential orientation, even on highly-ordered substrates.

11:20am **BI-TuM10 A Multi-Parameter QCM Technique for Investigations of Protein and Surface Interactions**, ***F. Höök****,* Chalmers Univ. of Tech. and Göteborg Univ., Sweden*.; M. Rodahl,* Q-Sense AB, Sweden*; B. Kasemo,* Chalmers University of Technology, Sweden

Protein molecules in contact with solid, non-biological materials, is a situation of broad scientific interest and technological importance, and there is a growing need for new tools to study these interactions. For instance, if the influence from the surface is large enough, the conformational-free-energy minimum for a protein attached on a surface might correspond to a conformation that differs from that of the native protein. It is thus likely that a protein-surface interaction might affect the conformation and hence the function of the proteins. We have developed a sensor system based on the traditional quartz crystal micro balance (QCM) technique, but where both the resonant frequency (f) and the energy dissipation (D) are measured simultaneously for a non-driven (freely oscillating) sensor crystal. This offers a possibility to investigate changes in the viscoelastic properties of adsorbed proteins in real time, which are further directly related to the conformation of the adsorbed proteins. Examples of how this type of measurements contributes with such information are presented using examples of: (i) Hemoglobin (Hb) adsorbed with and without the ligand carbon monoxide, which is known to slightly effect the conformation and stability against denaturation of Hb in solution. (ii) Antibody-antigen reactions, where we emphasize the added value from this type of multi-parameter analysis for immuno-sensing or of recognition events in general. (iii) Adsorption and enzymatic induced cross-linking of a mussel adhesive protein. We also demonstrate how additional information about these and similar types of measurements are obtained by simultaneously also measure at different frequencies, since different conformational states in some situations respond differently at different frequencies. We also demonstrate the importance of multi-parameter analysis in order to be able theoretically treat the QCM response upon adsorption of non-rigid biomolecules.

11:40am **BI-TuM11 Probing the Oxidation of Amine Modified Surfaces by MALDI Mass Spectrometry**, ***G.R. Kinsel****, R.B. Timmons, A.K. Walker, Y. Wu,* University of Texas, Arlington

The oxidation of amine modified surfaces, produced by pulsed RF plasma polymerization of allyl amine, can lead to substantial changes in the interaction of these surfaces with peptides and proteins in solution. Initial studies, using matrix assisted laser desorption / ionization (MALDI) mass spectrometry to characterize surface-peptide retention affinity, suggest that theses changes result from the acquisition of significant acidic character by the surface during the oxidation process. We have undertaken a variety of studies designed to characterize the surface chemical changes resulting from exposure of amine modified surfaces to air and to quantitate the impact of these changes on the peptide retention affinity. In these studies amine modified surfaces were exposed to pure oxygen and pure carbon dioxide environments. Time dependent changes in surface chemistry were monitored by FTIR spectroscopy and global compositional changes in surface chemistry were monitored by X-ray photoelectron spectroscopy. Subsequently, surface-peptide retention affinities were determined as a function of solution ionic strength and surface oxidation by using MALDI mass spectrometry. In addition, MALDI mass spectrometry was used to directly characterize oxidative changes in low duty cycle allyl amine polymer films to gain insight into the nature of the chemical modifications occurring in these polymer films. The results of these studies provide unique insight into the specific chemical changes and stability / reactivity of these surface modified materials.

Biomaterial Interfaces Group
Room 613/614 - Session BI-TuA

Characterization of Biomaterial Interfaces

Moderator: M. Grunze, University of Heidelberg

2:00pm **BI-TuA1 Surface Characterization of Biomaterials with Protein Layers**, ***H.J. Griesser***, CSIRO Australia, Australia **INVITED**

In the fabrication and interfacial analysis of novel biomaterials and their biological interactions, vacuum-based methods occupy a prominent role. Much research centers on the fabrication of "hybrid" biomaterials, which comprise a synthetic carrier material and an immobilized layer of biologically active molecules. Low temperature gas plasma methods are well suited to the attachment of reactive chemical groups onto polymers. Alternatively, plasma polymer interlayers can be used to provide reactive surface groups for the covalent interfacial immobilization of proteins. Vacuum-based surface analysis techniques characterize the surface properties of a material and increasingly are applied to the study of interfacial interactions with biological molecules. In this talk I will present examples of recent work on the immobilization of proteins and synthetic peptides on polymers via plasma polymer interfacial bonding layers. Detailed, multitechnique characterization of surface derivatizations and protein immobilizations is essential since proteins can adsorb and thereby mimic an intended covalent immobilization. Intended attachments are first modelled using derivatization reactions, and the surface density of reactive groups is thus determined. MALDI mass spectrometry is uniquely suited to the detection of adsorbed biomolecules at amounts much below monolayer coverage, and this method has been used to distinguish between covalent and adsorptive immobilizations. MALDI-MS is also eminently suited to the study of which proteins adsorb from complex, multicomponent media. For instance, the ways in which different surface chemistries of contact lenses influence which proteins adsorb onto lenses worn by human volunteers, has been characterized by MALDI-MS, and this information is being used for the guided design of improved coatings. Finally, I will discuss how AFM in the force mode provides complementary information to vacuum-based analysis methods.

2:40pm **BI-TuA3 Surface-plasmon Field-enhanced Fluorescence Spectroscopy and -Microscopy for the Evaluation of the Hybridization Reaction of Oligonucleotides**, ***W. Knoll***, Max-Planck-Institut für Polymerforschung, Germany and Stanford Univ., Germany; *D. Kambhampati, T. Liebermann, T. Neumann,* Max-Planck-Institut für Polymerforschung, Germany

Surface plasmon spectroscopy (SPS) is widely used as a surface-sensitive technique to characterize thin film architectures, or to monitor kinetic processes like biorecognition and binding events or photo-reactions in these layers. We describe an extension of the method combining the field-enhancements obtainable at the resonant excitation of surface plasmons with fluorescence detection schemes. Controlling the balance between the evanescent character of the surface mode and the energy (Förster) transfer between the chromophores and (the acceptor states of) the metal substrate sensitivity enhancements of more than 2 orders of magnitude compared to SPS can be achieved (though not label-free). We demonstrate the potential of this mode of operation for the quantitative evaluation of hybridization reactions between surface-immobilized probe oligonucleotides (15-mers) and complements from solution. It is shown that a simple Langmuir adsorption/desorption model describes the experimental results. Single base mismatches can account for a decrease in the equilibrium constant by two orders of magnitude, a second mismatch can give a reduction by another 3 orders. A further extension that will be introduced is the simultaneous observation of several hybridization/dehybridization reactions on a 3 x 3 matrix of 9 different sensor spots by fluorescence microscopy.

3:00pm **BI-TuA4 Characterization of Supported Biomimetic Films Using Broadband Vibrationally Resonant Sum-Frequency Generation**, ***K.A. Briggman***, *T. Petralli-Mallow, L.J. Richter, A.L. Plant, J.C. Stephenson,* National Institute of Standards and Technology

Supported organic films have received considerable attention as model biological membranes, as well as biomolecular templates for the development of biomimetic devices. A complete characterization of these biomimetic films requires the application of in-situ techniques, capable of probing fully hydrated systems. We have been exploring the potential of broadband vibrationally resonant sum frequency generation (SFG) as an in-situ probe for the study of hybrid bilayer membranes (HBMs). Our novel broadband approach@footnote 1@ provides a complete SFG spectrum over a window of several hundred wavenumbers, combining interface sensitivity and molecular specificity with the advantages of short acquisition times and no need for wavelength tuning. We have acquired vibrational SFG spectra of a variety of supported biomolecular compounds, including phospholipids in HBMs. A discussion of the preparation and stability of the HBMs as examined by SFG will be presented. @FootnoteText@ @footnote 1@Vibrationally resolved sum-frequency generation with broad-bandwidth infrared pulses, Opt. Lett. 23 1594 (1998).

3:20pm **BI-TuA5 Biosensors in Biomaterials Research**, ***K.I. Lundström***, Linköpings Universitet, Sweden **INVITED**

There are several surface physical tools, which are used to study the interaction between biomaterials and tissue. Many of them require special sample preparation and can not be used to follow the kinetics of interaction at the biomaterial surface. They can thus not be considered for in vivo applications either. Biosensor technologies developed for the monitoring of biomolecular interactions and utilizing (surface) physical phenomena as the detection principle should, however, be suitable for kinetic studies both in vivo ant in vitro. In this contribution some of the biosensing technologies for biomolecular interactions with and at surfaces are described, with special attention to surface plasmon resonance instrumentation and quartz crystal microbalances. It is concluded that by modifying the surface of such biosensors it is possible to study several important phenomena related to biomaterials and biocompability. Furthermore it is concluded that biosensors can be used also to monitor parameters outside the biomaterial itself, such as coagulation factors, inflammatory mediators etc. Biosensors for in vivo studies of biomaterials are also touched upon. The present use of biosensors for biomaterials related research is reviewed. A few examples from studies of e.g. plasminogen bleeding surfaces, complement activation and blood coagulation at surfaces are given. Finally some future possibilities of surface oriented biosensors for biomaterial research are speculated upon. This includes for the elucidation of the behavior of (single) cells adsorbed on or interacting with biomaterial surfaces.

4:00pm **BI-TuA7 Direct Probing of the Surface Ultrastructure and Molecular Interactions of Living Microbial Cells with Atomic Force Microscopy**, ***Y.F. Dufrene***, *C.J.P. Boonaert, P.G. Rouxhet,* Universite Catholique de Louvain, Belgium

Understanding biointerfacial phenomena such as cell aggregation and cell adhesion requires knowledge of the surface structure and physico-chemical properties of living cells with a nanometer scale resolution. In this work, atomic force microscopy (AFM) was used to determine, in physiological conditions, the ultrastructure and molecular interactions at the surface of living spores of Phanerochaete chrysosporium and their changes during germination. Cell immobilization was achieved by mechanical trapping in porous membranes. High-resolution images recorded on dormant spores showed that the surface was uniformly covered with a regular pattern of rodlets. These structures were several hundreds nm in length and had a periodicity of about 10 nm, in excellent agreement with freeze-etching characterization. Force-distance curves recorded between a silicon nitride probe and the spore surface showed no adhesion forces upon retraction. Dramatic changes of cell surface ultrastructure and molecular interactions occurred during germination. Germinating spores had a very smooth surface, partially covered with granular structures which were the residues of the rodlet layer. Force-distance curves recorded on smooth areas showed strong adhesion forces. These are attributed to binding of polysaccharides, which have been detected by X-ray photoelectron spectroscopy (XPS) and considered to be responsible for spore aggregation. The approach presented here offers new possibilities for probing the local surface properties of prokaryotic, animal and plant cells in the native state.

4:20pm **BI-TuA8 Contact Mechanical Properties of Confined NIPAM Films at the Biomaterial Interfaces**, ***R. Luginbuehl***, *M.D. Garrison, Y.V. Pan, R.M. Overney, B.D. Ratner,* University of Washington

Smart polymeric materials, which change their structural properties upon stimulation, are of highest interest for industrial applications in surface coating and printing, sensor technology, biotechnology, medicine, and biomaterial research. Progress in precision engineered surfaces for biosensor applications strongly depend on appropriate techniques to analyze surfaces at the micro and nanometer level. Recently, considerable research effort has focused on the investigation of co-polymers and grafted polymers containing N-isopropylacrylamide (NIPAM). These polymers can

be engineered to undergo thermally induced structural and mechanical phase transitions around 32 ÂºC, which is drawn by hydrophobic forces and hydrogen bonding. The structural phase transition is accompanied by a change in volume, and therefore a change in mechanical properties, as well as a change in the surface free energy. We carried out scanning force microscope (SFM) investigations on surface confined NIPAM films. Thin films (thickness < 10 nm) were obtained by polymerization on selected substrates. Novel SFM techniques permit the observation of the transition behavior at the nanometer scale. Photolithographically patterned thin films were used to isolate changes in the polymer structure relative to a reference substrate. Contact mechanical properties, volume transition, and the interfacial energy were monitored as a function of the system temperature. The introduced SFM technique offers a unique combination of microscopy with spectroscopic analysis of surface interactions and local subsurface structural properties.

4:40pm **BI-TuA9 Novel Biomaterials through Tailoring of Solid Surfaces***, **J. Rühe,*** Max-Planck-Institute for Polymer Research, Germany

The modification of materials by monolayers of polymers, which are covalently attached to the surface of the substrate, is a very attractive way to improve the properties of solids in bio-oriented applications. We describe several new pathways for the synthesis of surface-attached ultrathin polymer films, which carry functional groups relevant for biological or biomedical applications. The polymer molecules are either grown at the surface of the substrate in situ by using self-assembled monolayers of initiators or preformed polymers are (photo-)chemically attached to the material, which is to be modified. Additionally, the formation of ultrathin, patterned networks of functional polymers will be described. Examples for groups contained in the monolayers are peptide moieties, which could act as cell recognition sites and DNA fragments for biochip applications. The characterization of the monolayers, especially the swelling of the layers in an aqueous environment, will be described.

5:00pm **BI-TuA10 Photoisomerization and Photo-induced Alignment of Ordered Polymer Ultrathin Films Containing DNA and Polypeptide Layers: Possibilities for Optobioelectronic Substrates***, **R.C. Advincula,*** University of Alabama at Birmingham, US*; Y. Wang, E. Fells, E. Wallace,* University of Alabama at Birmingham

Alternate polyelectrolyte deposition (APD) is a relatively new technique for fabricating multilayer ultrathin polymer films. Since the polymers are adsorbed from solution, it opens up the possibility for incorporating biological macromolecules such as proteins, or nucleic acids in the active site that may be interesting for biosensing or biocompatibility issues. In the case of DNA molecules, selectivity arises from the interaction with various specific DNA reagents such as intercalators and DNA-complementary (hybridization) interactions. The multilayer complex films are also good model systems to investigate interaction between polynucleotides and polypeptides. The advantage of ultrathin film geometries in substrate supported systems is that they allow direct structural analysis using X-ray, FT-IR, SPS, etc. on a number of substrates, e.g. ITO-, Gold- coated glass, Si, etc. In this work, we report the formation of highly ordered ultrathin films containing DNA and/ or Polylysine/ Azobenzene dye multilayers fabricated using the alternate polyelectrolyte deposition (APD) approach. An important modification is the incorporation of photoisomerizable azobenzene dyes in the films to explore the possibility of using these films for some optobioelectronic applications. The photoisomerization of the dyes were investigated with respect to thickness, irradiation parameters, pair combinations, etc. The formation of polypeptide-dye multilayer complexes resulted in photo-induced circular and linear dichroism. This was investigated using polarized UV-vis spectroscopy, ATR, and SPS configurations. The conformation of the polypeptide and the DNA was investigated by FT-IR. In-situ adsorption experiments were investigated using ellipsometry and QCM.

Biomaterial Interfaces Group
Room 4C - Session BI-TuP

Poster Session

BI-TuP1 Characterization of Adsorbed Protein Films by Static Time of Flight Secondary Ion Mass Spectroscopy (ToF-SIMS), ***M.S. Wagner***, *J.B. Lhoest, D.G. Castner,* University of Washington

The development of effective biomaterials requires knowledge about the composition and structure of the adsorbed protein film that is formed upon implantation of a biomedical device in the body. Static Time of Flight Secondary Ion Mass Spectroscopy (ToF-SIMS) is a powerful technique for probing the molecular structure and composition of the outermost layers of a surface, making it an ideal technique for characterizing adsorbed protein films. Previous static ToF-SIMS studies of adsorbed protein films have shown the data obtained are complex fragmentation patterns arising from the amino acids present in proteins. To efficiently utilize all the information in these complex spectra requires multivariate analysis methods. We have used Principle Component Analysis (PCA) and Partial Least Squares (PLS) regression of the static ToF-SIMS data to characterize the composition of adsorbed protein films on mica and Teflon surfaces. PCA was used to differentiate the pure films of various plasma proteins (albumin, fibronectin, fibrinogen, etc.). PLS regression was used to quantitate the surface composition of protein films resulting from the competitive adsorption of binary protein mixtures comprised of albumin/fibronectin and albumin/Immunoglobulin G. For the albumin/fibronectin mixtures it was found that solutions containing more than 20% fibronectin produced adsorbed protein films that appeared to be pure fibronectin. The static ToF-SIMS results could be quantified by calibrating them with radiolabeled protein adsorption results. The overall aim of this research is to generate of a ToF-SIMS spectral database that can be used to determine the composition and structure of multicomponent adsorbed protein films with static ToF-SIMS.

BI-TuP2 Immobilization of Proteins on Self-Assembled Monolayers by Electrostatic Interactions, ***Y. Du***, *S.S. Saavedra,* University of Arizona

Immobilization of proteins onto solid substrates has been studied widely because of the importance of biomolecular films molecular devices. Adsorption of protein molecules onto solid surfaces by physical interactions is technically simple immobilization methods. However, in order to form highly ordered protein films, a single type of site-directed binding must be employed. In this study, sulfonated silane films were prepared on Si substrates. The resulting anionic surface was used to electrostatically adsorb positively charged proteins, such as horse heart cytchrome c. The formation of the sulfonated silane films was supported by XPS measurements, contact angle measurements, and ellipsometry measurements. The nature of the electrostatic adsorption of protein molecules and the protein film structure were investigated using a variety of surface spectroscopic techniques.

BI-TuP3 Adsorption Studies of Barnacle Cement Proteins from Balanus Improvisus, ***K. Mjorn***, *F. Hook, H. Elwing,* Goteborg University, Sweden

Marine specimens such as barnacles and mussels anchor themselves to solid surfaces with biological glues that are curable under water. The glue used by barnacles is called cement, which is a clear proteinous liquid. When a barnacle is dislodged from a surface under certain conditions, such cement is secreted. The adsorption of fresh cement from the barnacle Balanus improvisus without prior purification has been studied on an electrically inert non-polar methylterminated thiolated gold surface and polar gold surface by a combined use of Surface Plasmon Resonance (SPR) technique and an extended version of Quartz Crystal Microbalance (QCM-D) technique. With SPR, which is an optical surface sensitive method, we can determine the adsorbed amount of protein at the surface due to the difference in refractive index between water and the protein. With QCM-D, an acoustic method, we can determine the degree of bound water in the protein layer and the structural flexibility of the proteins. The adsorbed mass was almost twice as great for the hydrophilic gold surface compared to the mass adsorbed on the hydrophobic surface showed with SPR. The amount coupled water did not differ in a great extent between the two surfaces. This indicates that the flexibility and the amount adsorbed water did not vary much with the chemistry of the surface, in contrast to the mussel adhesive protein, Mepf-1, showed by C. Fant et al.@footnote 1@ The behavior of the cement monolayer could then be followed with these two methods when the buffer conditions where changed, e.g. ionic strength and pH. The influence of this was also followed with 2D SDS-PAGE, where the separation of fresh cement was compared to treated cement. How and at which circumstances the cement is cured is under investigation. @FootnoteText@ @footnote 1@C. Fant, K. Mjorn, H. Elwing and F. Hook, Adsorption behavior and enzymatic induced cross-linking of mussel adhesive protein (submitted).

BI-TuP4 Soft X-ray Spectromicroscopy Studies of Protein-Polymer Interactions, ***A.P. Hitchcock***, *T. Tyliszczak, Y.M. Heng, R. Cornelius, J.L. Brash,* McMaster University, Canada*; H.W. Ade,* North Carolina State University

Soft X-ray microscopy and micro-spectroscopy has considerable potential for characterizing the interaction of biological molecules with the surfaces of polymers used for biomaterials applications such as blood contact. We are exploring the use of scanning transmission X-ray microscopy (STXM) and various surface-sensitive X-ray microscopies - photoelectron emission microscopy (PEEM), scanning photoelectron microscopy (SPEM), and electron yield detection in STXM - for characterizing phase segregation at surfaces of polymers used for biomaterials applications, and for investigating specificity of cell and protein interactions with polymer surfaces. Transmission mode (STXM) has been used to study multilayer albumin adsorption on a polyurethane thin film which had micron-scale phase segregation. C 1s and N 1s STXM images and spectra recorded before and after protein exposure demonstrate that C 1s absorption spectroscopy differentiates protein, hard segment (urea), and soft segment (polyether) polyurethane signals and can locate proteins relative to an underlying phase segregated polymer. The strong C 1s -> @pi@*(C=O) excitation at 288.3 eV was found to be sufficiently well separated from the spectral features of the underlying polyurethane that considerable sensitivity to protein is achieved. The results clearly demonstrate that NEXAFS microscopy is relevant to biomaterials problems and that spatial segregation of protein does occur on phase separated polymers. Results to date suggest that albumin prefers the aromatic hard segment rather than the aliphatic soft segment regions of polyurethanes. The status of efforts to develop surface sensitive detection using total electron yield in STXM will also be discussed. X-ray microscopy is carried out at the Advanced Light Source (supported by DoE under contract DE-AC03-76SF00098), supported financially by NSERC (Canada).

BI-TuP5 A Circulating Flow Surface Plasmon Resonance System for Measuring Protein-Vesicle Binding Affinities, ***J. Shumaker-Parry***, *L.S. Jung, M.H. Gelb, C.T. Campbell,* University of Washington

We have developed a circulating flow surface plasmon resonance (SPR) system for measuring equilibrium binding of proteins to surface-bound vesicles. Traditional methods for measuring interfacial binding of proteins to membranes often require the use of a non-physiological reporter group such as a tryptophan residue. The addition of a tryptophan has been shown to perturb binding in some cases. The fluorescence-based methods also lack the sensitivity to measure high binding affinities (K@sub d@ < 10@super-7@M). Other methods that rely on centrifugation are plagued by protein loss through non-specific adsorption on tube surfaces. Our circulating flow SPR system measures equilibrium binding constants rapidly without a spectral probe and can be used to quantify values of K@sub d@ down to 10@super -10@M. The sensing surface contains a high density of vesicles immobilized via biotinylated lipids that bind to biotin sites in a streptavidin monolayer. Fluorescence studies using dye-filled vesicles show that the vesicles remain intact when attached to the surface. We have used the surface-bound vesicles to measure K@sub d@ values for phospholipase A@sub 2@ with phospholipid vesicles. A circulating flow system is used to introduce the protein to the vesicle surface and the protein concentration can be adjusted by adding protein or buffer to a reservoir. A binding isotherm is generated by performing equilibrium titrations and is used to obtain the K@sub d@ value directly without the need to fit association and dissociation rate curves. We have shown that vesicles provide a more biologically relevant surface for measuring binding affinities than planar hybrid bilayer membranes.

BI-TuP6 Adsorption Behaviour and Enzymatic Induced Cross-linking of the Mussel Adhesive Protein, Mefp-1, ***C. Fant***, *H. Elwing, F. Hook,* Goteborg University, Sweden

The blue mussel produces a family of 3, 4-dihydroxyphenylalanine (DOPA) containing proteins that allow the organisms to attach themselves to solid surfaces with high adhesive strength. We have studied the adsorption behaviour of the mussel adhesive protein, mefp-1, to a non-polar CH@sub 3@-terminated thiolated gold surface and to a polar silicone dioxide (SiO@sub 2@) surface. Cross-linking of these monolayers was induced by

catechol oxidase, which catalyses the transformation of DOPA to a highly reactive o-quinone participating in cross-linking. The adsorption and the cross-linking of mefp-1 was studied with respect to changes in the mass and viscoelastic properties of the protein adlayer. This has been possible by combined use of the Surface Plasmon Resonance (SPR) and an extended version of the Quarts Crystal Micro Balance (QCM-D@super TM@). The SPR response are proportional to the adsorbed mass (m). QCM-D measures change in mass uptake as a change in frequency (f) of a piezo electric quartz crystal. The change in frequency is proportional to the adsorbed mass including trapped water. In addition, the QCM-D technique allows simultaneous measurement of the energy dissipation (D) related to the rigidity/viscoelasticity of the adlayer. The results suggest that the protein layer formed on the polar surface is rigidly attached. In contrast, the layer formed on the non-polar surface is flexible, and it contains a large amount of bound water. Upon cross-linking, the dissipation decreased more on the non-polar surface compared to the hydrophilic surface. This demonstrates that the combination of these techniques provides unique information with respect to rigidity and water content of the adsorbed protein. The combination of these two techniques also make it possible to study the cross-linking of mefp-1, which is important knowledge for the development of a medical glue.

BI-TuP7 Thin Films of Functionalized Poly(Ethylene Gylcol) for the Specific Attachment of Proteins, ***S.N. Xia***, *H.B. Lu, C.T. Campbell, D.G. Castner,* University of Washington

The formation of ultrathin monolayers (10-30 angstroms thickness) of functionalized poly(ethylene gylcol) (PEG) oligomers and PEG-grafted siloxane polymers on gold surfaces has been investigated using x-ray photelectron spectrsocopy (XPS), static time of flight secondary ion mass spectromety (ToF SIMS), and surface plasmon resonance (SPR). PEG chains (MW 2000 to 5000) with an orthopyridyl-disulfide (OPSS) terminal group could be directly attached to a gold surface via formation of a gold-thiolate bond. By varying the type of terminal group at the other end of the PEG chain, the PEG monolayer can inhibit protein adsorption (OCH3), selectively attach proteins with surface cysteines (OPSS) through the formation of disulfide bonds, or selectively attach proteins with surface lysines (N-hydroxysunccinimide, NHS) through the formation of amide bonds. PEG monolayers that inhibit protein adsorption can also be formed from adsorption of siloxane polymer chains grafted with both methoxy-terminated ethylene gylcol and dialkyl disulfide side chains onto gold surfaces. The ability of the polymeric monolayers to specifically attach proteins with suface lysines was accomplished by replacing some of the methoxy-terminated PEG side chains with NHS-terminated PEG side chains.

BI-TuP8 A Novel Method for Characterization of Cell Surface Interactions, ***S. Kihlman,*** Chalmers University of Technology, Sweden*; C. Fredriksson, M. Rodahl,* Q-Sense AB, Sweden*; D.M. Steel,* Göteborg University, Sweden*; B. Kasemo,* Chalmers University of Technology, Sweden

A real-time in vitro technique to probe cell-surface interactions at biomaterial interfaces (e.g., contact surface, adhesion proteins, membrane and cytoskeleton modifications of the cell), would be very useful. In this study, we have explored the potential of a new method to characterize cell-surface interactions in liquid environments by dynamically monitoring the Quartz Crystal Microbalance (QCM) response due to adhering cells. The method is based on a new technique of simultaneously extracting resonant frequency and dissipation factor of the QCM. This is called QCM-D. It has here been applied to study the adhesion process of Chinese hamster ovary cells to polystyrene surfaces in serum-containing and serum-free environment at 37°C. The results show that: (i) adhesion of small colonies of a few hundred cells can readily be detected and characterized using the QCM-D technique, and (ii) the method of combining QCM data from changes in resonant frequency and dissipation factor gives new, relevant information about the character of the cell adhesion on surfaces and appears to be sensitive to properties of the surface (such as wettability) as well as the presence of serum-proteins on the surface.

BI-TuP9 The Role of Angiogenesis at Biomaterial Interfaces, ***P.C. Stephans,*** University of Washington*; R.B. Vernon, E.H. Sage,* Hope Heart Institute*; P.S. Stayton,* University of Washington

When a biomaterial is implanted, a relatively avascular fibrotic layer forms around the device. For devices such as sensors, this can inhibit the proper function of the device because the sensor only samples the microenvironment of the capsule. However, if the vascularity of the tissue adjacent to the device is increased, then the sensor can continue sampling a more representative environment. This problem has led to an interest in angiogenesis, the growth of new blood vessels from an existing vasculature, and how it is altered by various material properties. We are studying three material factors that may affect angiogenesis. The first, porosity, is based upon previous in vivo work that demonstrated a correlation between porosity and the number of vascular structures located close to the material. The second is coating the material with molecules, such as extracellular or matrix proteins. For example, we are currently immobilizing a peptide from SPARC that has shown angiogenic activity in vivo. The third factor is the release of soluble agents in the context of a material. Various soluble factors have been identified that are angiogenic, but now we can study the factor release from a material to determine if the angiogenic response can be stimulated within the material. To facilitate these studies, we are developing in vitro assays to study angiogenesis in the context of a biomaterial. For example, we have developed an assay to measure migration, a crucial step in angiogenesis. A teflon fence is used to grow endothelial cells to confluence in a well-defined area on the material. Once the fence is removed, the migration on various surfaces can be measured. The macrophage, a key player in angiogenesis, can also be incorporated into the assay to determine the effect of cytokine secretion on the endothelial cells. By studying the materials with these assays, we hope to gain a better understanding of the cell-material interactions that guide angiogenesis.

BI-TuP10 A Biosensor for In Vitro Monitoring of Cancer Cell Adhesion Behaviour, ***G. Nimeri,*** Gothenburg University, Sweden*; C. Fredriksson,* Q-sense AB, Sweden*; R. Hultborn, H. Elwing,* Gothenburg University, Sweden

A quartz crystal microbalance and dissipation (QCM-D) sensor, allowing simultaneous resonant frequency (f) and energy dissipation (D) measurements, was used to monitor cancer cell adhesion behaviour (attachment, spreading, and death) in vitro. This method is a mechanical sensor based on a minute (1nm) oscillation in the ultrasound frequency range. By measuring changes in f, the technique can be used to monitor the contact area of the cells with the substrate. Changes in D, which reflect energy losses as a result of friction in the system, provide information related to the internal structure (e.g., stiffness of the cytoskeleton). These two parameters offer new real time information regarding the status of cultured cells in vitro without interruption. The QCM-D signals were monitored for FADU cancer cells (human carcinoma squamous cells) for 8-24 hours. Cells were injected into a specially designed measurement chamber, filled with a minimal essential buffer and kept at 37 degrees C. The deposition and consecutive behaviour on the sensor surface, pre-coated with a tissue culture quality polystyrene overlayer, was then followed. Cell numbers from a few thousand up to a hundred thousand cells on a 1 cm2 surface were studied. The results show that the cells adhere and form a settled layer on the surface with relatively stable baselines in f and D after 4-8 hours. These baseline values indicate that a cultured layer of cells would provide contributions in f and D which can be monitored e.g., during cell growth or treatment. Preliminary results on the adhesion behaviour of non-treated and cells irradiated with 4 Gy (normal tumor treatment) show that the signals are distinctly different. The indications of differences in behaviour are considerably earlier than current methods based on growth rates (DNA staining etc).

BI-TuP11 The Effects of Surface Chemistry and Adsorbed Proteins on Monocyte/Macrophage Adhesion to Surfaces, ***M. Shen***, *T.A. Horbett, Y.V. Pan, B.D. Ratner, K.D. Hauch,* University of Washington

Adherent macrophages play a central role in inflammatory responses to implanted biomaterials. Human monocyte adhesion to surfaces was therefore studied to determine the effects of surface chemistry, adsorbed proteins, and adhesion time. The surface chemistry of a series of commercially available modified polystyrene (PS) surfaces, fluorinated ethylene-propylene polymer (FEP), and plasma-polymerized-tetraglyme (PPT) coated FEP was analyzed by ESCA. The surfaces were preadsorbed with plasma, serum, fibrinogen, fibronectin, or albumin. Human monocytes in 10% serum were allowed to adhere to the surfaces for 2 hours or 1 day. Adhesion was measured by an LDH method. After 2 hours, monocytes adhered to most surfaces under all conditions examined. Adhesion was greater on charged hydrophilic TCPS, Plastek C, or Primaria than on hydrophobic PS, Plastek A, Plastek B, or FEP. Adhesion was lowest on uncharged hydrophilic PPT-coated FEP or Costar's Ultra Low Attachment hydrogel, which were also shown to resist fibrinogen adsorption. Monocyte adhesion was greater on surfaces preadsorbed with fibrinogen or fibronectin than on surfaces preadsorbed with albumin. However, 2-hour adhesion to surfaces preadsorbed with serum was similar to surfaces preadsorbed with plasma, despite the lack of fibrinogen. Preadsorption of dilute plasma or serum increased adhesion to TCPS but did not increase adhesion to PS. After 1 day, monocyte adhesion decreased and was lowest

to surfaces without preadsorbed proteins. One-day adhesion was greater on plasma than on serum preadsorbed surfaces and was much greater on fibrinogen or fibronectin than on albumin preadsorbed surfaces. Overall, monocytes adhered to all surfaces and preadsorbed fibrinogen and fibronectin significantly promoted monocyte adhesion. Non-fouling surfaces that minimize protein adsorption may minimize overall macrophage adhesion and activation.

BI-TuP12 Human Monocyte/Macrophage Adhesion to Biomaterials Surfaces, ***J. Shan***, *T.A. Horbett, C.M. Giachelli,* University of Washington

The biocompatibility of synthetic implants is thought to be related to their attractiveness to monocyte/macrophages. The hydrophobicity and protein adsorption capabilities of an implant surface are believed to influence the adhesion of monocytes and macro phages. Therefore, a range of materials varying widely in hydrophobicity and protein adsorption characteristics were evaluated for monocyte/macrophage adhesion in vitro. Freshly isolated human peripheral blood monocytes were cultured on surfaces in the p r esence of autologous serum from 2 hour up to 8 days. The surfaces includes polystyrene (PS), tissue culture treated polystyrene(TCPS), human fibrinogen (Fbg) preadsorbed to PS (Fbg-PS), BSA preadsorbed PS (BSA-PS), plasma polymerized hexaflurocarbon (C 3F6) coated PS, plasma polymerized triglyme coated PS(triglyme), fluoro-ethylene-propylene (FEP) copolymer, and plasma polymerized tetraglyme coated FEP(PEO-FEP). Monocyte adhesion was determined by measuring the LDH activity of lysates. Of these surfaces, t he most hydrophobic is C3F6 coated PS, the most hydrophilic surface was PEO-FEP, which had less than 5ng/cm2 adsorption of Fbg from 1% human plasma. Adhesion to most PS based surfaces was similar for periods up to 24 hours. However, monocyte adhesion to T CPS was much lower by 24 hours. Monocyte adhesion to PEO-FEP at earlier hours was about 1/2 to 1/3 of the level achieved on PS and FEP, respectively. After the 8 day period, macrophage adhesion to the surfaces ranged from none on TCPS and Fbg-PS, low on B SA-PS, to significantly higher on PS, C3F6, triglyme and PEO-FEP. Adhesion to PEO-FEP after the 8-day period was similar to that on PS, C3F6, and triglyme, however macrophage morphologies differed among surfaces. Possible explanations (including non-protein mediated mechanisms) for monocyte/macrophage adhesion to PEO-film will be included in the poster presentation.

BI-TuP13 Adhesion of Endothelial Cells to Patterned OTS Surfaces, ***S.R. Webb***, *T.B. Boland, D.R. Weinbrenner,* Clemson University

Thromboresistance is necessary for a biomaterial in vascular applications to maintain proper function. One approach to thromboresistance is to line the interior wall of artificial vascular grafts with endothelial cells.This study examines the effects of surface chemistry and adhesion of endothelial cell membranes to proteins. Hence, understanding these two factors will facilitate applications to reduce thrombogenesis. Cell response to patterned materials was examined by employing highly organized monolayers of self-assembled octadecyltrichlorosilane (OTS) on a silicon wafers. Pure OTS monolayers are poor substrates for cell growth, most likely because of the denaturing of serum proteins near the surfaces. The OTS surfaces were exposed to an electron beam, which selectively oxidized the organic film, creating a variety of oxygenated species at the surface. The monolayers and the patterned surfaces were analyzed by ellipsometry, water contact angle, electron spectroscopy for chemical analysis (ESCA) and AFM. Bovine heart endothelial cells were cultured. Cells were inoculated at 1 x 106 cfu/mL and cultured in twelve well plates in the presence of pure and patterned OTS surfaces. To insure the cells were not toxic to the OTS surfaces three cytotoxicity test were performed. The first was a live dead assay in which calcein green was used to determine if the cells were viable. The second test was MTS to determine if the cells were proliferating. The last test to determine how cytotoxic the surfaces were to the cell was BCA or total protein test. All three tests proved that the patterned surface was not toxic to the cells. The attachment, spreading and growth of the cells on the surfaces will also be presented. The cell spreading on the etched surfaces suggests that the cells may be able to attach more firmly to the extracellular proteins on the etched pattern. The results from this cell growth study will aid in designing micro-patterned surfaces for cell-based biosensors.

BI-TuP14 Microcontact Printing of Protein Patterns to Direct Cellular Response, ***T.C. McDevitt***, *M.D. Garrison, T.R. Kyriakides, M. Scatena, P. Bornstein, C.M. Giachelli, P.S. Stayton,* University of Washington

In order to control the cellular response at a biomaterials interface, we are applying micropatterning techniques to spatially arrange extracellular matrix (ECM) ligands on surfaces. Cell function has previously been related to cell shape and cytoskeletal architecture, thus we are investigating how microcontact printing of proteins can be used to modulate these parameters. Various combinations of ECM proteins can be patterned to create heterogeneous, ordered surfaces that selectively promote cell adhesion, spreading, and intracellular signaling events. Designated adhesive and non-adhesive regions are dictated by the choice of particular ECM proteins which possess these characteristics and cell attachment is confined by the features of the micropatterning. Our aim is to use micropatterning as a tool to recreate an ordered, complex surface that more accurately resembles the true ECM, stimulates a specific and desirable cellular response, and thus induces a more favorable interaction with the biomaterial.

BI-TuP15 Surface Functionalization Strategies for Miniature Multichannel Biosensing, ***H.B. Lu***, *J. Homola, C.T. Campbell, B.D. Ratner, S.S. Yee,* University of Washington

Biosensor development has advanced towards highly integrated, multichannel array configurations with more detection power and faster speed. Robust surface functionalization methods for immobilizing sensing molecules and making non-fouling surfaces are needed. Also, precise spatial control of surface functionalization is valuable. In this presentation, various surface functionalization methods including orthogonal self-assembly (OSA), masked plasma polymerization and protein contact printing are introduced. The potential of these methods for precisely immobilizing sensing biomolecules to specific surface regions, or creating a non-fouling area using passivation molecules, is discussed. We used a dual-channel surface plasmon resonance (SPR) sensor with a thin tantalum oxide (Ta2O5) overlayer configuration for demonstrating the feasibility of these surface functionalization methods, as well as for further developing a miniaturized multichannel biosensor. The high refractive index dielectric Ta2O5 overlayer covers part of the gold surface to excite part of the incident light in a higher wavelength and thus produce a second SPR dip besides the original gold SPR dip. To use this second SPR dip as an internal reference channel, the surface functionalization methods were used to deliver biosensing molecules (e.g. antibodies) or passivation molecules (e.g. oligo(ethylene glycol) or bovine serum albumin) to the gold or the Ta2O5 surface respectively. The optical nature of such an SPR sensor configuration requires precise delivery of molecules to these two surface regions in order to separate signal from the two channels. Therefore, it provides an excellent tool for demonstrating the spatial control ability of the surface functionalization methods used. The surface functionalization strategies described in this paper should have general applicability for developing miniaturized multichannel biosensors in other formats as well.

BI-TuP16 Kinetics of Vesicle Adhesion and Fusion, ***K. Glasmästar***, *F. Höök, C.A. Keller,* Chalmers University of Technology and Göteborg University, Sweden*; V.P. Zhdanov,* Chalmers Univ. of Tech., Göteborg Univ., Sweden and Institute of Catalysis, Novosibirsk, Russia, Sweden*; B. Kasemo,* Chalmers University of Technology and Göteborg University, Sweden

Vesicle adhesion and fusion are essential in many cellular processes and in the formation of supported membranes. Because of their similarity to natural membranes, they play an important role in the development of biosensors and in model studies of membrane-mediated processes. We have studied the kinetics of adsorption of small unilamellar lipid vesicles on carefully prepared SiO@sub 2@ surfaces and their subsequent fusion to form a lipid bilayer, using surface plasmon resonance (SPR) and a new quartz crystal microbalance (QCM-D) technique. With the latter the energy dissipation (D) and the resonance frequency (f) of the QCM oscillator are measured. The lipid mass adsorbed at the surface and the mass of water trapped by the adsorbed layer are measured as changes in f. The SPR technique is only sensitive to the amount of lipid adsorbed. Since one of the primary differences between lipids adsorbed as vesicles or as a bilayer is the water trapped within and between the vesicles, the combination of the two techniques provides a detailed picture of how a lipid bilayer forms on the SiO@sub 2@ surface. In addition the two types of adsorption have very different viscoelastic properties, which are reflected in the D factor. The formation of a lipid bilayer on a SiO@sub 2@ surface is a two-stage process. First a layer of intact vesicles adsorbs on the surface. Then at a sufficiently high surface concentration the vesicles begin to break and form a fluid bilayer. From detailed QCM-D and SPR measurements at different vesicle concentrations in the liquid phase, we find that (i) the adsorption is irreversible, (ii) it is rate limited by bulk diffusion, (iii) vesicle to bilayer transformation starts at a critical surface coverage, after which (iv) further adsorption from the bulk phase drives the vesicle to bilayer transformation. The kinetics of these events is further elucidated by Monte Carlo simulations, employing different mechanistic models for the kinetics.

BI-TuP17 Thiopeptide-Tethered Lipid Bilayers for the Incorporation of the Enzyme Complex Cytochrome c Oxidase, ***H.D. Lauer,*** Max-Planck-Institut for Polymer Research, Germany*; E.K. Schmidt,* Lab. for Exotic Nanomaterials Frontier Research Program, Japan*; R. Naumann,* Max-Planck-Institut for Polymer Research, Germany*; A. Offenhäusser,* Max-Planck-Institut for Polymer Reseach, Germany*; W. Knoll,* Max-Planck-Institut for Polymer Research, Germany

Lipid bilayers form the basic structure of biological membrane due to which membrane proteins are able to carry out their specific functions. Membrane proteins have precisely designed moving parts whose mechanical actions are coupled to chemical events. This coupling of chemistry and movement is the reason why membrane proteins play the predominant role in most biological processes. Without a better understanding how proteins operate, it is very difficult to apreciate cell biology. Therefore in the last few years a number of model systems have been developed to create a biomimetic system. To retain the functionality of an incorporated membrane protein, the lipid bilayer should be fluid and the membrane/protein-complex has to be to some extent spatially decoupled from the substrate. Without this decoupling from the surface, the protein denatures. A number of spacers are well known to decouple the membrane/protein-complex from the gold substrate and preserve a thin water reservoir between lipid and the gold.@footnote 1, 2, 3@ We present here a biomimetic system utilising thiopeptides as spacer molecules chemisorbed onto a gold surface and covalently attached to lipid layer.@footnote 4@ This system is used to incorporate the membrane protein cytochrome c oxidase, a key enzyme in the cell respiration. Optical and electrochemical characterisation methods are used to obtain more information about the architecture and the operation of the membrane/protein-complex. @FootnoteText@ @footnote 1@ Cornell, B.A. et al., Nature, 387, 1997, 580 @footnote 2@ Vogel, H. et al., J. Phys. Chem., 99, 1995, 1038 @footnote 3@ Evans, S.D. et al., Langmuir, 13, 1997, 751 @footnote 4@ Naumann, R., et al., Angew. Chem., 107, 1995, 2168.

BI-TuP18 Functionalized Glycolipids for Model Biomembrane, ***S. Schiller,*** Max-Planck-Institut for Polymer Research, Germany*; H. Kunz,* University of Mainz, Germany*; W. Knoll,* Max-Planck-Institut for Polymer Research, Germany

Fluid Model Biomembranes are important tools for general investigations of membrane properties and have many potential applications in the pharmaceutical industry e.g. for analyte screening using competitive immuno assays with membrane-integral receptors. The most advanced of these systems are tethered lipid bilayer membranes (tBLMs) on gold or silicon. tBLMs developed so far often show poor electrical properties compared to BLMs. Some systems insufficiently decouple the lipid bilayer from the surface and do not provide optimal tether properties in terms of hydrophilicity of the submembrane and stable membrane support, which is necessary for the incorporation of membrane proteins. An alternative route uses functionalized carbohydates as tether molecules to meet some of these requirements. Several carbohydrate systems are already under investigation, polysaccharides, especially dextran, cellulose and agar are used to provide a hydrophilic, soft and gel like support. Regio- and stereospecific manipulations in small and defined areas are difficult in such large polymer systems. We therefore focus on small oligosaccharides and their functionalized derivatives and the synthesis of a complex multicomponent system with specific lateral spacer molecules and several glycolipid tether sytems. Important components are glycolipids functionalized with anchor groups for surface attachment and spacer saccharides. The possibility to introduce side functionalities to multifunctional saccharides opens a wide range of variations, for example the functionlization with fluorescent probes at different positions along the tether chain or variation of physical properties by varying side groups. The approach we present here consists of the use of several saccharides and different lipids to learn more about the fundamental properties of these systems. Further research will be the synthesis of the complex system described above and the challenging study of complex membrane processes.

BI-TuP19 Force Differentiation Assay: A New Approach Sensing, ***M. Natesan,*** GeoCenters*; C. Yanavich,* Nova Research*; S. Metzger,* Geocenters*; G.U. Lee,* Naval Research Laboratory

Biomolecular interactions have recently been measured at the single molecule scale using microscopic techniques such as atomic force microscopy, optical tweezers and micropipettes. The force required to rupture a specific interaction has been found to be a characteristic of the structure of the molecular interaction and the rate at which it is stressed. We have used these insights to develop a new approach to molecular detection based on sensing force. Force detection has two advantages, it has single molecule sensitivity and the magnitude of the force can be used to differentiate specific from nonspecific interactions. We will describe two force based diagnostic techniques that apply a magnetically derived force to an antibody-antigen sandwich using a micron size particle.

BI-TuP20 Solid Supported Lipid Bilayers by Fusion of Mixed Thiolipid/Lipid Vesicles onto Gold, ***H. Wieder,*** *S. Lingler,* Max-Planck-Institute for Polymer Research, Germany*; J. Mack,* Institute for Organic Chemistry, Germany*; A. Offenhäusser,* Max-Planck-Institute for Polymer Reseach, Germany

Recently, planar solid supported lipid membranes have become a frequently used model system for biological membranes. Their big advantage to other model systems is the relatively high stability to mechanical and electrochemical stress in addition to the accessibility by a variety of surface sensitive techniques. These features make them an interesting system for biosensing applications. In this work supported lipid mono- and bilayers tethered to Au-electrodes by thiolipids are being investigated using surface plasmon resonance spectroscopy (SPR), impedance spectroscopy (IS), cyclic voltammetry (CV), reductive desorption and contact angle measurements. Following work on hybrid bilayer membranes,@footnote 1@ we are now looking at systems with and without laterally diluted hydrophilic spacers of different length to provide a hydrophilic reservoir between membrane and support. The dependence of the membrane properties on different parameters in the preparation by self-assembly, LB-techniques and vesicle fusion are being investigated. Into the membranes noted above transmembrane proteins are reconstituted and checked for their functional activity. @FootnoteText@ @footnote 1@Lingler S., Rubinstein I., Knoll W., Offenhäusser A. (1997) Fusion of Small Unilamellar Lipid Vesicles to Alkanethiol and Thiolipid Self-Assembled Monolayers on Gold, Langmuir 13, 7085-7091.

BI-TuP21 Incorporation of Membrane Proteins into Lipid Bilayers Supported on a SiO@sub 2@-surface., ***A. Persson,*** *F. Höök, J Rydström, B. Kasemo,* Chalmers Univ. of Tech. and Göteborg Univ., Sweden

Transmembrane proteins are difficult to study in their native state, since detergents are used for the solubilization of transmembrane proteins, which might influence the protein properties and function. One way to circumvent this is to incorporate the proteins into lipid membranes deposited on solid supports. The focus of this project is to combine spontaneous formation of lipid membranes on solid supports, known to occur on SiO@sub 2@, with incorporation of transmembrane proteins. Understanding and mastering of this process has important implications for the development of biosensors and biomaterials, for investigations of the respiratory chain, studies of the photosynthesis, and in neurobiology. Small unilammellar vesicles (SUV's) form a lipid bilayer on hydrophilic SiO@sub 2@-surfaces.@footnote 1@ This process is most likely a two-stage process, where initially intact vesicles adsorb at the surface at low coverages. When a certain surface-concentration of SUV's is reached, the vesicles break and form a fluid bilayer. The protein containing SUV's studied in this work seem to behave in the same way. The SUV's were prepared by sonicating different phospholipids in buffer, and the proteins were incorporated with detergent. The vesicle and protein adsorption is studied with a new QCM-D technique where the frequency shift (mass adsorbed on the surface) and the energy dissipation shift (reflecting the viscoelastic properties of the overlayer) are measured simultaneously.@footnote 2@ The presence of the proteins in the supported bilayer is, after deposition, directly measured using the QCM-D technique, combined with a secondary process using specific monoclonal antibodies to the membrane bound proteins. The preparation procedures and the first results are presented. @FootnoteText@ @footnote 1@Keller, C. A., et al. 1998. Biophys. J. Vol 75 p.1397 @footnote 2@Rhodahl, M., et al. 1995. Rev. Sci.Instrum. Vol 66 p.3924.

BI-TuP22 A Dissimilatoric Nitrate Reductase as a Signal Transducer in a Tethered Membrane FET Architecture, *H. Borcherding,* ***Th. Hettmann,*** *S. Diekmann, P. Steinruecke,* IMB Jena e.V., Germany

Nitrate reductase is a key enzyme in the anaerobic denitrification of nitrate to elementary nitrogen. Nitrate is reduced to nitrite in the catalytical @alpha@-subunit of the enzyme. The dissimilatoric enzyme from Pseudmonas stutzeri also comprises a membrane-integral @gamma@-subunit which is involved in the transfer of electrons from the the bacterial quinole pool to the site of nitrate reduction. This makes the enzyme a valuable functional unit for reconstitution in lipid bilayers. We developed a suitable purification protocol for nitrate reductase from Ps. stutzeri . The enzyme is reconstituted in phospholipid vesicles which are used for the

reconstitution of the enzyme in a tethered bilayer covering the gate of a field effect transistor. By generation of a nitrate-specific transmembrane potential, it should be possible to design a nitrate-specific FET biosensor. Data will be given on the suitability of our approach to create a new type of biosensor.

BI-TuP23 On Compressional Wave Velocity in Animal Bone by Compensated Ultrasonic Timer Technique (CUTT), ***S. Mohiuddin***, King Saud University, Saudi Arabia

Ultrasonic propagation properties play a vital role, as they throw light on molecular architecture and cellular assembly of the living system, in understanding the physiological reality of the system which is involved in the life processes. The present investigation on compressional wave velocity of cancellous bone (Scapula and rib) and compact bone (femur) by adopting compensated ultrasonic timer technique, reveals that in cancellous bone tissue (scapula and rib), ultrasonic compressional wave velocity is the same, but it is relatively more than that of compact bone (femur). Considering the values of compressional wave velocity obtained for scapula, rib and femur, when measured at different places of the same sample along its axis, there exit no definite relations between the parameters related to the composition the bone and the parameters concerned with the propagating ultrasonic wave in the tissue. The compressional wave velocity in bovine scapula, rib and femur bones is more than those reported for soft tissues and hard calcified derivatives of integuments. It is interesting to note that the velocity in the bone is high when compared to other tissue of the same animal.

Biomaterial Interfaces Group
Room 613/614 - Session BI-WeM

Cell Solid-Surface Interactions

Moderator: W. Knoll, Max Planck Institute for Polymer Research, Germany

8:20am **BI-WeM1 Fabrication of Biologically Active Interfaces upon Self-Organization of Amphiphilic Polymers**, ***T. Nishikawa***, *J. Nishida, K. Nishikawa, R. Ookura, S.-I. Nishimura, S. Wada, T. Karino, H. Okubo, M. Matsushita, S. Todo, M. Shimomura,* Hokkaido University, Japan

Fabrication of cell culture substrates possessing micro surface morphology is one of the current topics in biomaterial research. Recently we found that two dimensional honeycomb structures can be fabricated by casting dilute solutions of amphiphilic compounds on solid supports in a humid atmosphere. The structural feature of the honeycomb films is a two dimensional single layer of hexagonally arrayed holes, whose diameter is ranging from 1 μm to 10 μm. We suggest that the honeycomb films work as artificial basal films - biologically active interfaces between cells and solid supports. The porous structure will enable the adhered cells to reach and interact with the surface of solid support as well as the exposed surface of the cast films. The pore size, porosity, and thickness of the films can be major factors which control the cell behavior on the culture substrates. In this sense cell behavior on porous surfaces can be influenced by the surface morphology as well as the chemical properties of the polymers constituting the films. In this report we describe the fabrication of the honeycomb films and the cell culturing on the films from the view point of factors affecting the cell adhesion in detail. Honeycomb films with various pore size and film thickness were fabricated by casting dilute solution of amphiphilic copolymers on water surfaces. The films were transferred onto cell adhesive supports (slide glass) or non-adhesive supports (polyhydroxyethylmethacrylate coated glass plate). Bovine aorta endothelial cells or hepatocytes were cultured on the honeycomb films. The thicknesses of the films were varied with the water temperature of the subphase (at 6°C 0.2 μm in thickness and 4 μm in hole diameter and at 20°C 1.5 μm in thickness and 4 μm of hole diameter). The cell adhesion to the honeycomb films was considerably influenced by the film thickness, which determines the distance between the adhered cells and the solid supports.

8:40am **BI-WeM2 Reactions of Biological Cells to Nanostructures**, ***A.S.G. Curtis***, *C.D.W. Wilkinson,* University of Glasgow, Scotland

The reactions of biological cells to nanostructured polymer and silica surfaces will be described. The surfaces have been prepared in a variety of materials using E-beam methods, colloidal resists and replication by embossing. The reactios include changes in adhesion and cytoskeletal organisation. The effects of topography in the ranges 20-100nm greatest plan dimension, 50-200nm repeat and 10-100nm height will be described. The degree to which the cells conform to these surfaces will be reported with details of the closeness of approach of the cells to the surfaces. The question of whether the reactions are to topography, chemistry or surface disorder at boundaries will be discussed.

9:00am **BI-WeM3 Engineering Cell Surface Chemistry**, ***C.R. Bertozzi***, University of California, Berkeley **INVITED**

Many important biological processes are initiated by cell surface molecules, such as cell-cell adhesion and communication during development, virus-host cell binding, tumor cell metastasis and immunological recognition. Consequently, the ability to chemically control the display of epitopes on cell surfaces would enable a myriad of possibilities for studying cell-cell interactions and for engineering cells with novel properties. This presentation will focus on work in my laboratory that aims to apply the principles of organic chemistry to orchestrating cell surface chemistry. We have harnessed the cell's metabolic machinery to remodel cell surfaces with reactive organic functional groups. The foundation of our approach is the unnatural substrate tolerance of several enzymes involved in oligosaccharide biosynthesis, which permits the conversion of unnatural monosaccharide precursors into cell surface-associated oligosaccharides. We have exploited these pathways as vehicles for the delivery of uniquely reactive electrophilic functional groups, such as ketones and azides, to cell surfaces. For example, we demonstrated that an unnatural analog of N-acetylmannosamine bearing a ketone group, N-levulinoylmannosamine (ManLev), is metabolized by human cells to N-levulinoyl sialosides on the cell surface, resulting in the cell surface display of ketone groups. The cell surface can then be selectively reacted with rationally-designed organic structures bearing a complementary nucleophile such as an aminooxy group which reacts to form a stable covalent adduct. The ability to engineer chemical reactivity into endogenous cell surface molecules suggests many potential applications including the engineered adhesion of cells to materials and artificial surfaces. @FootnoteText@ @footnote 1@ Mahal, L. K.; Yarema, K. J.; Bertozzi, C. R. Engineering Chemical Reactivity on Cell Surfaces Through Oligosaccharide Biosynthesis. Science 1997, 276, 1125-1128. @footnote 2@ Mahal, L. K.; Bertozzi, C. R. Engineered Cell Surfaces: Fertile Ground for Molecular Landscaping. Chemistry & Biology 1997, 4, 415-422. @footnote 3@ Lemieux, G. A. ; Bertozzi, C. R. Chemoselective Ligation Reactions with Proteins, Oligosaccharides and Cells. Trends Biotech. 1998, 16, 506-513. @footnote 4@Yarema, K. J.; Mahal, L. K.; Bruehl, R.; Rodriguez, E. C.; Bertozzi, C. R. Metabolic Delivery of Ketone Groups to Sialic Acid Residues. Application to Cell Surface Glycoform Engineering. J. Biol. Chem. 1998, 273, 31168-31179. @footnote 5@ Lemieux, G. A.; Yarema, K. J.; Jacobs, C. L.; Bertozzi, C. R. Exploiting Differences in Sialoside Expression for Selective Targeting of MRI Contrast Reagents. J. Am. Chem. Soc. 1999, 121,4278-4279.

9:40am **BI-WeM5 Biocompatibility of Cardiac Cells on Silane-Modified Surfaces**, ***J.J. Hickman***, *M.S. Ravenscroft, H. Canavan,* The George Washington University*; V. Krauthamer,* Food and Drug Administration

We are investigating the interaction of cardiac cells with select silane-modified surfaces in a defined culture system. Our goal is to examine cell reaction in vitro to the types of modified surfaces that may be useful as implant coatings with an assay that we could relate to organ function. The SAM-modified glass coverslips were analyzed using X-ray Photoelectron Spectroscopy (XPS). The morphology was determined by image analysis and the excitability of the cultured cardiac cells was examined electrophysiologically by Calcium imaging both at rest and while being stimulated. Stimulation experiments electrically pace the cells at select time intervals and monitor the resulting changes in intracellular [Ca]. We observed a significant difference in excitation-induced Ca changes on the different silanated surfaces without corresponding differences in cell morphology. This result implies a change in cardiac Ca channel function on SAMs with different functional groups that would not be detected by morphological analysis alone.

10:00am **BI-WeM6 Biomaterials That Talk**, ***P.S. Stayton***, *T.C. McDevitt, K.E. Nelson, C.M. Giachelli,* University of Washington*; R.B. Vernon, H. Sage,* Hope Heart Institute*; D.G. Castner,* University of Washington

We are working to develop a variety of biomaterials that are designed to communicate with biomolecules, cells, and tissues. This goal requires complementary surface assembly techniques, engineering of biomolecules designed for surface applications, and detailed characterization of the biomolecules at surfaces. In this talk, I will present joint protein engineering and microfabrication approaches to constructing coatings that control cell phenotype in confined dimensions on device surfaces. These coatings are designed to interact with specific receptors to control cell motility, proliferation, and protect against apoptosis.

10:20am **BI-WeM7 Biofunctionalization of Surfaces with Peptide Amphiphiles**, ***M. Tirrell***, University of Minnesota, U.S. **INVITED**

Peptides carry enormous capacity and versatility for participating in specific ligand-receptor binding interactions. As small fragments of proteins, they offer the possibility of delivering a selected activity in constructing a biofunctionalized surface or interface, absent other, undesired activities present in the full protein molecule (e.g., immunogenicity). We have been exploring the self-assembly and cell recognition properties of peptide fragments (thus far derived from extracellular matrix fragments) that we have lipidated synthetically by attaching a phospholipid-mimic, double-chain, hydrocarbon tail. Lipidation confers interesting amphiphilic and self-organization properties on the molecules and enables the stable deposition of layers of peptide amphiphiles on surfaces. Specifically, we have been using peptide amphiphiles to functionalize surfaces with peptide fragments derived from collagen and fibronectin. Deposition of these molecules by Langmuir-Blodgett methods gives a very high degree of control over the density and orientation of the surface molecules. This in turn enables us explore the effects on cell response of peptide density and molecular architecture variations with a great degree of precision. The principal results so far, which seem to have some generality for different kinds of peptides, are that there is an optimum peptide density for each kind of peptide fragment, and that the architecture of peptide presentation is a very sensitive controller of bioactivity. Examples will be given of these effects.

11:00am **BI-WeM9 Plasma Copolymer Surfaces for Cell Culture**, ***R.D. Short***, University of Sheffield, UK

The plasma copolymerisation of a functionalised monomer with a hydrocarbon diluent comonomer can be used to fabricate surfaces of controlled chemistry. By employing low plasma power, fragmentation can be kept to a minimum and the functional group preserved from the functionalised monomer to the plasma copolymer deposit. This has been demonstrated for carboxyls, carbonyls, alcohols and amines. Changing the ratio of functionalised monomer to hydrocarbon monomer allows films of varying functional group concentration (functional groups per 100 carbons) to be prepared. Substratum surface chemistry is known to play a critical role in the attachment, spreading and proliferation and differentiation of cells in tissue culture. The culturing of different cell types - keratinocytes (primary), endothelial cells (cell line) and osteoblasts (primary and cell line) - on plasma copolymer films containing carboxyl, carbonyl and alcohol functionalities has been investigated. Cell response has been explored with functional group concentration. Optimum attachment, spreading and proliferation were obtained on surfaces containing carboxyl groups - these surfaces contained 3-5 carboxyl groups per 100 carbons. Actin-staining by direct immunofluorescence was used to visualise changes in osteoblast cytoskeleton with substratum chemistry. It was observed that as the substratum carboxyl concentration increased cell spreading was notably enhanced. As few as 5 carboxyls per 100 carbons were sufficient to support good cell attachment and a well-defined polygonal cell morphology on an essentially hydrophobic surface.

11:20am **BI-WeM10 Mechanical Properties of a Bone Marrow Cell-Knit Composite for Tissue Engineering: Evolution under Mechanical Load**, ***B. Müller***, *G. Ettel, D. Siragusano, T. Brandsberg, F. Brandsberg, M. Petitmermet, A. Bruinink, J. Mayer, E. Wintermantel,* ETH Zürich, Switzerland

Knitted textiles provide a 3D scaffold for optimal spatial and nutritional conditions in engineering biological tissue in vitro. The vital-avital composite formed by the textile fabric and the ingrown cells can be stimulated by mechanical load. Introduced by cyclic stretching it affects the proliferation and differentiation of bone cells as indicated by specific protein synthesis and cell mass increase. As an additional parameter, the evolution of the mechanical properties of the vital-avital composite is in situ measured by a piezoelectric force sensor. The system for the stimulation of in vitro cell cultures is calibrated by the use of a coil spring minimizing frictional losses. Reference measurements are performed using multifilament PET-knits as untreated ones and others saturated with serum proteins. After autoclaving and under constant load, both types of knits show an exponential run-in behavior with a time constant of about 2h. In the frequency range investigated (0.1 to 3.0Hz) the amplitude raise lies between 10 and 15%. Long-term experiments (5 days) with cyclic mobile and immobile phases of 3 and 6 hours, respectively exhibit a linear decrease of 5% in amplitude for the protein saturated knits, however. Finally, the preliminary experiments using primary adult rat bone marrow cells demonstrate that the stiffness of the vital-avital composite increases by a power law as a result of mechanical stimulation (Stretching is as low as 2%). Therefore, the successive force measurements reflect the physiological mechanical state of the cells and, consequently, enable optimizing the properties of the forming tissue. Determinants are the cell density, the stretching, the frequency of mechanical excitation, and the time span for mobile and immobile phases. A mathematical model developed on the basis of nonlinear mesoscopic elasticity theory describes the experimental observations.

11:40am **BI-WeM11 The Effect of Lipopolysaccharide Structure and Composition on Microbial Cell Adhesion**, ***S. Kim***, *J. Curry,* University of Arizona

Lipopolysaccharide (LPS), the main component of the outer membrane of Gram negative bacteria, consists of a lipid component, termed lipid A, that anchors the LPS in the outer membrane, a sugar core, and a variable O-specific polysaccharide chain. Whenever a bacterium approaches a surface, LPS predominantly mediates the interaction because of its inherent location on the cell surface. Varying size and structure of LPS molecule depending on bacterial strain appears to be an important determinant of the overall charge and hydrophobic character of the cell surface. Furthermore, some workers have shown that change in its chemical composition or pattern lead to a dramatic change in its biological activity. Those facts suggest that studying adhesion as a function of LPS structure and chemical composition may help to better understand the mechanisms of bacterial adhesion. The overall goal of our research is to understand at the molecular scale how the structure and composition of the LPS affects bacterial adhesion and biological activity by direct force measurement using the Surface Forces Apparatus (SFA). Specifically, we will measure the force of interaction and adhesion between two hydrophilic (bare mica) and hydrophobic (surfactant coated) surfaces in the presence of LPS molecules at different environmental conditions (i.e. temperature, pH, ionic strength). Samples used in this study will be several rough mutant LPS (R-form) molecules of which structures are well characterized. Along with the knowledge of their structure and chemical composition, the complete force profile will allow us to better predict adhesive properties of several different types of bacteria. This work will be very meaningful for research in many areas where microbial adhesion is important, for instance, biofilm formation and microbial transport in porous media.

Biomaterial Interfaces Group
Room 613/614 - Session BI-WeA

Biology at the Nanoscale
Moderator: H.G. Craighead, Cornell University

2:00pm **BI-WeA1 Forces and Mechanisms of Self-Assembly: Non-Equilibrium and Transient Effects**, ***J.N. Israelachvili***, University of California, Santa Barbara, US **INVITED**

Recent experimental and theoretical work has provided new insights into the intermolecular forces and mechanisms involved in the self-assembly of biological structures such as protein complexes, vesicle aggregates and structured biological materials. It appears that many biological structures can only be formed sequentially in space and time, whereby different interactions and processes occur in different regions of space and at different times. The final structure or 'state' is therefore not the thermodynamically equilibrium state, but a 'transient' structure that nevertheless performs its allotted function at optimum efficiency - be it the opening of an adhesion site or conduction pore (with a lifetime of microseconds or milliseconds), a site-specific binding protein (with a lifetime of milliseconds or seconds), a drug-delivery carrier (with a lifetime of days), or a biomaterial with a lifetime of years. Examples will be given to illustrate the generality of such systems, and the implications of non-equilibrium and transient effects to self-assembly in vivo and in vitro will be discussed.

2:40pm **BI-WeA3 Pulling Protein Networks in 2D**, ***G. Baneyx***, *V. Vogel,* University of Washington

Fibronectin (Fn), an adhesion protein with multiple recognition sites, mediates cell attachment to synthetic and biological surfaces. In solution, Fn exists in a globular state where most of its recognition sites are buried in the protein core. Surface adsorption induces conformational changes in the protein that expose many of these sites. Furthermore, Fn assembles into detergent insoluble fibers on the surface of cells, and these matrices are considered to be the main functional form of the protein. The cell-mediated assembly of Fn into fibrillar matrices is a complex, multistep process that is still incompletely understood. This is due to the chemical complexity of the extracellular matrix, as well as a lack of experimental control over the molecular interactions and dynamic events. We will discuss how Fn fibril assembly into extended two-dimensional networks can be induced by adsorbing Fn from a physiological buffer solution to a dipalmitoylphosphatidylcholine (DPPC) monolayer. A sequential model for the assembly pathway is proposed. Striking similarities are found between the properties of these Fn fibrils assembled underneath DPPC monolayers and those found on cellular surfaces, as well as between the respective sequential assembly pathways. Spontaneous Fn fibril assembly underneath DPPC monolayers can now serve as a well controlled model system to study how different parameters such as the deletion of key Fn sequences, as well as alteration of solution conditions and the presence of other proteins, affect the molecular assembly pathway.

3:00pm **BI-WeA4 Separation of Long DNA in a Microfabricated Channel with Submicron Constrictions**, ***J. Han***, *H.G. Craighead,* Cornell University

Microfabricated fluid channels with submicron constrictions were used to separate long DNA molecules according to their sizes. The channels were fabricated on Si substrate by photolithography and reactive ion etching techniques, followed by an anodic bonding to make a sealed channel. These channels have alternating thick and thin regions, which allows long DNA molecules to relax during the electrophoretic motion.@footnote 1@ Separation was achieved by the difference in the probability for DNA molecules to escape these 'entropic traps'. Many fluorescence-labeled DNA molecules were collected at the first entropic trap, and launched simultaneously to form a band of DNA molecules. After traveling the channel, each DNA species formed a separated band, due to the mobility difference in the channel. The traveling time of the DNA bands through the channel were measured by monitoring the fluorescence intensity at the end of the channel. Several long DNA species (35~160kbps) were separated as bands in this way, typically within 30 minutes, which is significantly lower than pulsed field methods. Several structural parameters, such as the depth or the length of the thin and thick region, were varied to study the effect on the DNA mobility and the length range of molecules that a given device can separate. Once these relevant parameters are characterized, this device could be a fast way to separate DNA molecules and other polymers. @FootnoteText@ @footnote 1@ J. Han and H. G. Craighead, J. Vac. Sci. Tech. A, in publication (1999)

3:20pm **BI-WeA5 Engineered Nanostructures to Control Microtubule Motion Along Kinesin Tracks**, ***J.S. Clemmens***, *J.R. Dennis, J. Howard, V. Vogel,* University of Washington

Motor proteins such as kinesin have evolved to transport molecules over long distances along microtubules within cells. The objective of this study is to engineer molecular tracks of motor proteins to direct the motion of microtubules on nanoengineered synthetic surfaces. We have demonstrated that kinesin moves microtubules parallel to nanoscale ridges of shear-deposited poly(tetrafluoroethylene) (PTFE) films,@footnote 1@ presumably due to preferential adsorption of kinesin along specific topographical features. Additionally, we have observed that other proteins adsorb similarly to shear-deposited PTFE films. We aim to elucidate the molecular mechanisms of these phenomena in order to delineate design principles for engineering tracks of kinesin. To accomplish this aim, surfaces have been fabricated with well-defined nanoscale pits and grooves and systematically tested for their ability to preferentially adsorb proteins or motors from solution. Once important topographical features are identified, tracks following specified paths can be engineered. This is the first step in making molecular shuttles that can move, load, and unload cargo between user-controlled locations and against concentration gradients. In the future molecular shuttles may form the basis of transporting molecular cargo through synthetic matrices. @FootnoteText@ @footnote 1@Dennis, JR et al. "Molecular shuttles: directed motion of microtubules along kinesin tracks" Nanotechnology, in press. (1999)

3:40pm **BI-WeA6 Force and Compliance Spectroscopy of Single Peptide Molecule**, *M.A. Lantz, S.P. Jarvis,* ***H. Tokumoto***, JRCAT, Japan*; T. Martynski, T. Kusumi, C. Nakamura, J. Miyake,* NAIR, Japan

An exciting application of AFM to biology is to measure forces required to stretch and unfold individual molecule. This technique looks very promising for studying molecular structure. However, this work has been applied so far to large proteins with complex structures resulting from a variety of bonding mechanisms. This complexity makes the interpretation of the experimental results difficult. Hydrogen bonding plays a major role in the formation of the secondary and tertiary structures of polypeptides from which proteins are composed. Even though, the detailed energy landscapes involved in the formation of these structures are not well understood. Here we demonstrate a new experimental technique for performing single molecule AFM force spectroscopy on significantly smaller molecules than those previously reported. We have used this technique to study the mechanical properties of the synthetic peptide cystein3-lysine30-cystein, which we designed specifically to study hydrogen bonding. Under the experimental conditions used, the peptide adopts the a-helix structure as a result of hydrogen bonding within the molecule. Force-displacement experiments were used to measure the force (approximately 200 pN) required to stretch single peptides from the helical state into a linear chain and the measured force versus peptide elongation was used to calculate the work done in breaking the hydrogen bonds. The average experimental value of the hydrogen bond energy (20.2 kJ/mol) is in good agreement with reported theoretical calculations. In addition, we directly measured the stiffness of the molecule during elongation and found to vary from approximately 0.005 to 0.012 N/m.

4:00pm **BI-WeA7 Single Molecule Force Spectroscopy by AFM, Nanomechanics Meets Molecular Biology**, *K. Tolksdorf, M. Grandbois, M. Rief, H. Clausen-Schaumann,* ***H.E. Gaub***, Ludwig-Maximilians-Universität München, Germany **INVITED**

Recent developments in AFM-instrumentation allow the manipulation of single molecules and measurements of intermolecular as well as intramolecular forces. We took advantage of the high spatial resolution of the AFM and developed mechanical experiments with single macromolecules. An overview on this novel kind of spectroscopy will be given and applications in the field of polymer and life sciences will be highlighted: receptor ligand interactions were measured in single molecular pairs. Lateral distributions of interaction partners on samples were mapped in a chemical imaging mode. The length distribution of individual grafted polymers at surfaces was imaged. Individual polymers and proteins that were anchored on a gold surface were picked up with the AFM tip and stretched, their viscolelasticity and yield strength was measured. Proteins were reversibly unfolded and the conformation forces were determined at the level of single secondary structure elements. DNA double strands were stretched and unzipped. A model was developed based on elastically coupled two-level systems that allows the description of basic features of

the experimental results. @FootnoteText@ Rief, M.; Oesterhelt, F.; Heymann, B.; Gaub, H. E.Science 1997, 275, 1295-1298. Rief, M.; Gautel, M.; Oesterhelt, F.; Fernandez, J. M.; Gaub, H. E. Science 1997, 276, 1109-1112. M. Rief, H. Clausen-Schaumann , H.E. Gaub, Nature Struct. Biol. (1999), in press M. Grandbois, M. Beyer, M. Rief, H. Clausen-Schaumann ,H.E. Gaub, (1999),Science in press

4:40pm **BI-WeA9 Characterization of Antibody-Antigen Interaction Forces With Magnetic Tweezers**, *S. Metzger,* Geocenters*; M. Stevens,* Naval Research Laboratory*; G. Sagvolden,* University of Oslo, Norway*; C. Yanavich,* Nova Research*; M. Natesan,* GeoCenters*;* ***G.U. Lee****,* Naval Research Laboratory

Living systems have developed the capacity to use molecular interactions to control structure and function. In order to understand the molecular mechanism of these interactions, the force between and within individual molecules has been directly measured using microscopic techniques such as AFM and optical tweezers. We describe a technique for directly measuring intermolecular interactions using magnetic force. This technique allows intermolecular forces to be studied over an extended range of force (10@super-15@ to 10@super-15@ Newton) and time (1 ms to 10 sec). We will demonstrate the principles of this technique by measuring the force and time required to rupture antibody-antigen bonds.

5:00pm **BI-WeA10 Probing the Nano-environments of Peptides on Solid Surfaces by Advanced Secondary Ion Mass Spectrometry***, T. Schenkel,* Lawrence Livermore National Laboratory*; K.J. Wu,* Charles Evans & Associates*; A.V. Barnes, M.W. Newman, J.W. McDonald, A.V. Hamza,* Lawrence Livermore National Laboratory

The interaction and bonding of peptides and proteins in the solid phase and on solid surfaces is of central importance in biotechnological research. Embedding of analyte molecules in special matrix solutions (such as 2,5 dihydroxybenzoic acid) has been shown to produce enormous increases in yields of intact molecules both for laser and single charged ion induced ablation. The mechanisms responsible for this enhancement are however not well understood. Both the binding of matrix and analyte molecules in the solid and collisional ion formation processes have been suggested to play important roles. We have investigated the effect of sodium and potassium impurities on secondary ion emission from gramicidin S by time-of-flight secondary ion mass spectrometry (TOF-SIMS) with highly charged projectiles.@footnote 1@ Highly charged ions like Xe@super44+@ or Au@super69+@ increase secondary ion yields by over two orders of magnitude as compared to singly charged ions. Each highly charged projectile emits secondaries from an area of only about 10 nm^2. Analysis of coincidences among secondary ions detected following the impact of a single projectiles allows for the characterization of the nano-environment of e. g. a peptide molecule in a matrix solution. For the gramicidin S, we found that emission of Na@super+@ and K@super+@ ions was strongly correlated with emission of sodium and potassium adduct ions, [M+Na]@super+@, [M+K]@super+@. This correlation indicates the nestling of sodium impurities around peptide molecules. In our presentation we will discuss the potential of coincidence analysis in TOF-SIMS for the probing of nano-environments on surfaces of biomaterials. @FootnoteText@@footnote 1@A. V. Hamza et al., J. Vac. Sc. Technol. A 17, 303 (1999) Acknowledgement: This work was performed under the auspices of the U. S. Department of Energy by Lawrence Livermore National Laboratory under contract No. W-7405-ENG-48.

Biomaterial Interfaces Group
Room 4C - Session BI-WeP
Poster Session

BI-WeP1 Self-Assembly of Tetraphenylporphyrin Monolayers on Gold Substrates, ***A.L. Bramblett***, *M.S. Boeckl, T. Sasaki, B.D. Ratner, J.W. Rogers, Jr.,* University of Washington

The development of the next generation of medical implants involves attaching appropriate biorecognition molecules in the proper orientation and concentration on the surface of an implant, to prevent the cellular activation that leads to the foreign body response, and implant encapsulation. Porphyrin molecules are ideal for the development of a uniform monolayer, with controlled, optimal spacing of biorecognition groups. Self-assembled porphyrin monolayers on gold surfaces have been demonstrated with three custom synthesized alkylthiol substituted tetraphenylporphyrin molecules. Several techniques including x-ray photoelectron spectroscopy (XPS), ultraviolet/visible absorption spectroscopy (UV/Vis), scanning tunneling microscopy (STM), and grazing-angle infrared spectroscopy (GAIR) have been used to characterize the monolayers. XPS binding energy shifts in the S(2p) spectra reveal that the porphyrins are chemisorbed to the surface through a sulfur-gold bond.@footnote 1@ A red shift without a significant blue shift of the Soret band in the UV/Vis absorption spectra demonstrates that the porphyrin molecules are aligned on the gold surface in a side-by-side orientation.@footnote 2@ GAIR with a polarized light source, indicates that the porphyrin rings are oriented parallel to the gold surface. Round STM features, approximately 2 nm in diameter, correspond closely to the diameter of tetraphenylporphyrin (1.8 nm), and are distributed relatively evenly over the surface. Finally, XPS and UV/Vis coverage calculations show approximately monolayer coverage. Taken together, this data indicates the formation of self-assembled porphyrin monolayers. @FootnoteText@ @footnote 1@ Castner, D.; Hinds, K.; Grainger, D. W. Langmuir 1996, 12, 5083-5086. @footnote 2@ Osuka, A.; Maruyama, K. J. Am. Chem. Soc. 1988, 110, 4454-4456.

BI-WeP2 Protein Nanopatterning on a Gold/Aluminum Nanoarray, ***C.K. Woods***, *Z.-P. Yang, A. Chilkoti,* Duke University

Protein nanopatterning has potential applications in the fabrication of multianalyte, proximal probe biosensors, genomic arrays, as well as modulation of cell-substrate phenomena. We have developed a technique to immobilize proteins on a surface with spatial resolution of around 100 nm. An ultraflat nanoarray of gold and aluminum is created on a silicon wafer by combining nanosphere lithography with "ultraflat template stripping"- a technique for creating ultraflat thin films of metal. The ultraflat gold/aluminum nanoarray is then incubated in a hexadecanethiol (HDT) solution, which forms a hydrophobic, self-assembled monolayer (SAM) on gold but does not adsorb onto the hydrophilic, native oxide layer on aluminum. We hypothesized that protein adsorption on a HDT-functionalized gold/aluminum nanoarray should occur preferentially on the HDT SAM, thereby allowing proteins to be nanopatterned on the 100 nm gold features. Formation of the HDT SAM on gold but not on aluminum was investigated separately on gold and aluminum substrates using contact angle goniometry, ellipsometry and atomic force microscopy (AFM). The model protein, Ribonuclease A, was found to adsorb preferentially to the HDT SAM on gold with a signal to background ratio of about 6. AFM studies of protein adsorption on HDT-functionalized ultraflat gold/aluminum nanoarrays are currently in progress as are experiments on extending this approach using SAMs presenting biological ligands.

BI-WeP3 Osteoblast and Monocyte Response to Nanometre Surface Topography In Vitro, ***P. Hanarp***, Chalmers University of Technology, Sweden*; J. Rice, J.A. Hunt, J.A. Gallagher,* University of Liverpool, UK*; D.S. Sutherland, J. Gold,* Chalmers University of Technology, Sweden

It is well known that cells adhering to surfaces are influenced by micron-sized chemical and topographical features, but very little is known about cell behaviour on surfaces with smaller, nanometre-sized features. We have used a method based on adsorption of colloidal particles to produce surfaces with well-controlled nanotopography. Surfaces of silicon wafers, pre-coated with 30 nm of thermally evaporated Ti, were treated with aluminium chloride hydroxide giving a net positive charge at neutral pH. Then the surfaces were exposed to dilute aqueous solutions of 110 nm polystyrene particles, and the negatively charged particles adsorbed onto the positively charged surfaces randomly by electrostatic interactions. A submonolayer of particles was obtained with coverage controlled either by salt concentration in the colloidal solution for equilibrium adsorptions, or particle concentration and adsorption time in interrupted (non-equilibrium) adsorptions. To produce chemically homogenous surfaces, a film of titanium (82.5nm) was evaporated on top of the particle films. The titanium film was naturally oxidised in air. The response of primary human osteoblasts and monocytes to these surfaces has been investigated. The cells were cultured in contact with the samples, 1) flat titanium oxide, 2) 10% and 3) 20% coverage of 110 nm particles coated in titanium oxide, for 1 and 7 days. They were examined using fluorescent cytoskeletal staining, confocal microscopy and lactate dehydrogenase assays in conjunction with flow cytometry. At each time point, both osteoblasts and monocytes cultured on the flat titanium oxide and the 20% coverage surfaces showed a greater affinity for adherence than at the 10% surfaces. SEM analysis of the samples after cell culture showed that the surfaces are still intact. This work is ongoing, but these preliminary results indicate that osteoblasts and monocytes are influenced by nanotopography in vitro.

BI-WeP4 Gene Expression in Reaction to Micro and Nano-topography, ***R. Hartley***, University of Glasgow, Scotland, UK*; A.S.G. Curtis,* University of Glasgow, Scotland, UK, Scotland

Cellular reaction to surfaces has particular relevance to engineering tissue constructs. Advances in microfabrication enabling production of structures with defined surface topography have facilitated our understanding of cell elongation, orientation and movement. The vastly differing cell morphologies and cytoskeletal arrangement on planar and topographical surfaces necessitate an investigation of adhesion, signal transduction and transcriptional regulation. This work investigates gene transcription in reaction to micro and nano-topography. In this study we used two differing methods Differential Display RT-PCR to assess gene transcription. The first relies on large scale total RNA isolation following in-situ cell lysis or transferral to suspension and subsequent lysis. The second follows the Klebe method of RT-PCR without RNA isolation, where although RT-PCR is generally carried out and optimised for 250 cells, the protocol is suitable for four cells. This method has particular significance for analysis of gene expression on small areas where cell number is limited. Methods: Sub-confluent tendon epitenon were trypsinised and seeded directly onto topographic and control substrata. For large scale total RNA isolation directly in-situ, or in suspension, GITC phenol/chloroform was used and RNA equilibrated using 260/280nm absorbance. The Klebe method used a -70@super o@ C freeze/rapid-thaw in the presence of RNase inhibitor allowing time for cDNA library creation. In each case cDNA libraries were amplified by PCR in the presence of @super 32@P dATP, then denatured and run on a 7M urea, 5% acrylamide electrophoresis gel. Separated isotopically labelled ssDNAs were viewed by autoradiography.Phage display systems were also used.

BI-WeP5 Do Oligoethyleneglycol Terminated Alkanthiols Induce Complement Activation?, ***J.M. Benesch***, *S. Svedhem, S. Svensson, P. Tengvall,* Linköping University, Sweden

oligoethyleneglycol terminated alkanthiols with varying length of the ethylenglycol repeats were self assembled on gold surfaces. In Situ antibody ellipsometry techniques were used to study protein adsorption when the surfaces are incubated in serum or plasma. The results so indicate depositions of serum and also deposits of complement factors. This indicates that oligoethyleneglycol surfaces are not serum and plasma resistent. This behavior is dependent on the presence of calcium and the number of repeats in the ethylenglycol chain.

BI-WeP6 Hybridization of DNA Monolayers on Gold Observed In Situ with Surface Plasmon Resonance, ***G.B. Saupe***, *M.J. Tarlov,* National Institute of Standards and Technology

The hybridization of end-tethered single-stranded DNA (ssDNA) probes on gold surfaces with ssDNA targets in salt solutions was monitored with surface plasmon resonance (SPR). SPR is a sensitive technique for measurement of angstrom-level changes resulting from surface DNA hybridization reactions. Surfaces derivatized with DNA are of interest for a variety of applications including genetic diagnostics, forensics, and infectious disease detection. In this study we have used a model system of thiol derivatized ssDNA probes self assembled on gold. The surfaces were subsequently treated with mercaptohexanol to passivate regions of unreacted gold and eliminate non-specific binding of the DNA. The effects of target ssDNA length, base pair mismatches, and the locations of the matching sequence within the target were evaluated in high salt conditions (1M NaCl). Probe coverages, hybridization efficiencies, and times needed for hybridization were measured. Recent data for the hybridization of mixed target solutions, where single basepair mismatched targets compete

with exact sequence matched targets to hybridize a single probe, will be discussed.

BI-WeP7 Multivariate Comparison of Dodecanethiol Self-Assembled Monolayers Prepared by Microcontact Printing and Solution Assembly, ***D.J. Graham***, *D.N. Price, S.L. Golledge, M.D. Garrison, T.C. McDevitt, B.D. Ratner,* University of Washington

In this study we show how exploring the entire TOF-SIMS spectra using multivariate statistics can enhance the analysis of a surface and bring to light details that are not obvious from univariate analysis. We have applied a PCA analysis to TOF-SIMS spectra of SAMs of dodecanethiol on gold prepared by solution assembly (assembly time: t =2sec,1min,5min,15min,30 min,1hr,24hr,6d) and microcontact printing (stamping concentration: c =0.001,1,5,10,50,100,200,1000mMol). PCA models from the TOF-SIMS negative and positive data were constructed. ESCA composition scans were also taken of all surfaces. Both univariate and multivariate comparisons were made between the solution assembled and stamped samples. The SIMS ratio sum[I(MolecularIons)]/sum[I(C-C3hydrocarbons)] and the ESCA C/Au ratio were used to compare the samples. Both methods found good correlation between samples in which a stamping concentration of 10-50 mMol produced SAMs similar to fully solution-assembled SAMs. This data is consistent with results from a similar stamping study using STM and contact angles by Larsen et. al.@footnote 1@ PCA analysis provided deeper insight into the two sets of samples. The scores from the first principal component (PC1) showed the similarities between the samples prepared by the two methods, though there was more scatter in the stamped samples. The second principal component (PC2) showed that some differences exist between the two preparations. Loadings from the second principal component showed that the differences in the samples were due mainly to the presence of oxidized species and silicone contaminants. Only trace amounts of Si (<1%) were seen in the ESCA spectra on some samples. The increase in oxidized species in the stamped samples may be due to the stamping procedure that was carried out in air. These differences may not be detected by other methods or by univariate analysis. @FootnoteText@@footnote 1@N. B. Larsen, H. Biebuyck, E. Delamarche, and B. Michel, J. Am. Chem. Soc. 119, 3017-3026 (1997).

BI-WeP8 Observation of Metal Clusters on Pancreatic Cells by Lateral Force Microscopy, ***A. Cricenti***, Consiglio Nazionale Delle Ricerche, ITALY*; R. Generosi, S. Cotesta, M. Girasole, P. Perfetti,* Consiglio Nazionale delle Ricerche, Italy*; A. Congiu-Castellano,* Universita' di Roma La Sapienza, Italy

The interaction between pancreatic cells and metal ions have been studied at membrane level by Atomic/Lateral Force Microscopy in the repulsive regime of contact mode. The atomic force microscope can give both topographic and chemical (in lateral friction mode) informations on the cellular membrane of cells. Morphological characteristics of non infected cells and metal infected cells were easily imaged from fixed and dried cell preparations. Upon Cd2 and Zn ions uptake, the pancreatic cells don't change their morphology but the lateral friction images localized several metal clusters on the cellular membrane.

BI-WeP9 Microfabricated Cantilever Force Sensor for Measurement of Cell Locomotory Forces, *C.D.W. Wilkinson, A.S.G. Curtis,* ***B.W. Leslie***, University of Glasgow, Scotland

Microfabricated cantilever force sensors are potentially capable of measuring attoNewton (10E-18 N) forces. Here, we propose a design for such a force sensor capable of measuring forces involved in cell locomotion. The cell guidance structures and cantilevers were fabricated as one integrated unit using dry-etching of polysilicon. Substrate used was a 300 µm thick (100) oriented silicon wafer coated on both sides with a 50 nm thick layer of silicon nitride. Wafers were cut into 25 mm squares. A 10 µm thick layer of isotropic polysilicon was deposited on one side of the wafer square. After patterning, the polysilicon layer was dry-etched to a depth of 10 µm to form cell guidance structures and a row of fifteen cantilevers along two opposing edges of the wafer square. Controlled etching of the silicon nitride layer ensured that cantilevers were released from the substrate while cell guidance structures remained in place. Cell guidance structures are designed to steer the cells toward the free end of the cantilever where deflection is largest for a given force. Cantilever dimensions (length=900 µm, height=10 µm, thickness=1 µm) combined with optical lever sensing of cantilever deflection are theoretically capable of measuring forces as small as 3 pN.

BI-WeP10 Surface Characterization of Microfluidic Devices, ***H. Canavan***, *M.S. Ravenscroft, D. Ramaker,* George Washington University*; M.J. Tarlov,* National Institute of Standards and Technology*; J.J. Hickman,* George Washington University

Interest in the interactions of biomolecules with surfaces stems from various sources, including biocorrosion of ship hulls, the rejection of transplant materials in the human body, and biological fluids interactions with MEMS devices. A variety of chemical and physical factors affect biological fluid behavior in microchannels used in lab-on-a-chip devices. We report an investigation of the effect of polymer surface condition on microfluidic properties. The primary goal of this work is to correlate the electrophoretic flow properties through microfluidic channels of different polymer substrates with the surface condition of the polymers. While polymer substrates hold great promise for biological microfluidic applications, polymer surfaces are often poorly defined which can lead to irreproducible microfluidic behavior. To circumvent this problem, we are developing various protocols to control the surface functionality of polymers. Oxygen plasma treatment has been used to introduce oxygen functionalities that can then serve as active sites for covalent attachment of organosilane monolayers. XPS, SIMS, and contact angle measurements have been used to characterize as-is, oxygen plasma treated, and organosilane-modified surfaces of polystyrene, polymethylmethacrylate, and various co-polyesters. We will report on these measurements and preliminary fluid flow experiments.

BI-WeP11 Time-of-Flight SIMS Analysis of Micropatterned Biomaterial Arrays, ***S.L. Golledge***, *M.D. Garrison, B.D. Ratner,* University of Washington

Micropatterned arrays of thin films, self-assembled monolayers and biomolecules are a critical enabling technology for the development of miniaturized biosensors and next generation biomaterials. Accurate characterization of the locally defined surface functional states is therefore crucial to the successful implementation of these novel approaches to surface engineering. We employed Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS), in both spectroscopic (static) and imaging modes to define and identify the spatial presentation of (1) bioactive polymeric materials prepared via radio-frequency glow-discharge (RFGD), (2) non-fouling organic thin-films patterned through microcontact printing (uCP), and (3) direct uCP matrix proteins important in biological wound healing. TOF-SIMS allowed characterization of both the bioactive species and the engineered reference regions, in addition to assuring that unwanted contaminant species were not present. Future prospects for TOF-SIMS analysis of micropatterned arrays will be proposed.

BI-WeP12 Atomic Force Microscope and Surface Plasmon Resonance Investigation of Polymer Blends of Poly(Lauryl Methacrylate) and 2-Methacryloyloxyethyl Phosphorylcholine-co-Lauryl Methacrylate, ***S. Clarke***, *M.C. Davies,* University of Nottingham, U.K.*; V. O'Byrne,* Biocompatibles Ltd, U.K.*; C.J. Roberts,* University of Nottingham, U.K.*; J. Russell,* Biocompatibles Ltd, U.K.*; S.J.B. Tendler, P.M. Williams,* University of Nottingham, U.K.

In order to design new synthetic polymers for use in medical devices it is necessary to characterize the surface of the material to understand the interactions that occur when exposed to biological environments.@footnote 1@ Incorporation of phosphorylcholine (PC) into polymers has been shown to improve biocompatibility by suppressing unfavourable responses that occur on contact with tissue and bodily fluids.@footnote 2,3@ Polymers containing PC are currently in use as coatings for medical devices such as stents, catheters, or to fabricate contact lenses. As an alternative to synthesizing new PC-containing polymers, polymer blends offer the opportunity to investigate the surface properties of PC in new materials. Here, polymer blends of 2-methacryloyloxyethyl phosphorylcholine-co-lauryl methacrylate (PmMl@sub 6@) and poly(lauryl methacrylate) (PLMA) have been produced with varying ratios of the two components. The surface of the blends when coated onto silver has been characterised using X-ray photoelectron spectroscopy (XPS), tapping mode atomic force microscopy (TMAFM), and surface plasmon resonance (SPR). Analysis has revealed that the blends formed by the two polymers are immiscible and exhibit surface-segregation with nanometre-sized domains being formed throughout the range of the blends. The Pmml@sub 6@ is preferentially expressed at the surface of the blends leading to enhanced protein-resistant properties. @FootnoteText@ @footnote 1@ Davies et al, Chapter 4 in Biocompatibility:Assessment of Materials and Devices for Medical Applications. Braybrook, J (Editor) J. Wiley and Sons, 1997 @footnote 2@ Campbell et al (1994) Am. Soc. Artificial Organs J. 40 3 M853-M857 @footnote 3@ Ishihara et al (1991) J. Biomed. Mat. Res. 25 1397-1407.

BI-WeP13 Thermally Responsive Ultrathin Coatings by RF-Plasma Deposition, ***Y.V. Pan***, *R.A. Wesley, R. Luginbuhl, R.M. Overney, D.D. Denton, B.D. Ratner,* University of Washington

Poly(n-isopropyl acrylamide) (pNIPAM) shows a lower critical solution temperature (LCST) of 31@super o@C in aqueous environment. At temperatures lower than the LCST, the polymer chains are well hydrated and fully extended. The polymer chains dehydrate and take on a more compact configuration above the LCST. This interesting phase transition behavior has been observed here with pNIPAM chains grafted onto solid surfaces by plasma deposition. In this presentation, thermally responsive coatings are prepared f rom glow discharges of NIPAM vapor. The synthesis and spectroscopic characterization (XPS, SIMS) of plasma polymerized NIPAM (ppNIPAM) will be presented. The ppNIPAM coatings obtained showed a remarkable retention of the monomer structure, and a phase tr a nsition at 31@super o@C in both water and air. The phase transition was measured by a novel AFM method. The water phase transition was surprising because of the expectation that the plasma environment would destroy the specific NIPAM structure associat ed with t he thermal responsiveness. However, the transition in air was even more unexpected and suggests that adsorbed water on the AFM tip and at the polymer surface is sufficient to produce the phase change, even when the bulk of the polymer is unhydrated. Plasma polymerization of NIPAM represents a one-step method to fabricate thermally responsive coatings without specially prepared substrates and functionalized polymers.

BI-WeP14 Design of a Minimal Peptide for Adsorption to Hydroxyapatite and Cell Binding via an RGD Sequence, ***M. Gilbert***, *C.M. Giachelli, P.S. Stayton,* University of Washington

In the natural remodeling of bone, the proteins that comprise the extracellular matrix play keys roles in the signaling of bone cells. These ECM proteins contain amino acid sequences such as RGD which are important for the adhesion of bone cells to the protein coated bone surface as well as the transmission of signals via outside-in integrin pathways. Many of the ECM proteins also contain stretches of acidic amino acid repeats, gamma carboxyglutamic acid residues, or high degrees of sulfation, glycosolation, or phosphorylation which aid in the adsorption of the protein to the mineral surface of bone. Despite the large body of knowledge on bone biology, most modern bone implant designs do not employ any control over the degree or specificity of protein adsorption to the surface of the implant which can result in lack of integration of the implant or encapsulation of the implant. However, by understanding how the biomineralization proteins bind to mineral surfaces and transmit signals to bone cells, minimal peptides can be designed which contain the features of strong mineral adsorption as well as integrin mediated cell adhesion and intracellular signaling to improve implant integration. A minimal peptide based on the mineral binding motif of salivary statherin combined with an RGD sequence was designed to bind and orient on hydroxyapatite surfaces. This fusion peptide (called N15-RGD) is capable of binding with high affinity to hydroxyapatite with the same Langmuir parameters as just N15 as well as maintaining alpha helical content in solution. N15-RGD, while immobilized on HAP, is also capable of binding cells specifically and in a dose dependent manner via the RGD sequence. The main integrin responsible for the binding of the cells to the RGD sequence is the @alpha@@sub v@@beta@@sub 3@ integrin. The N15-RGD peptide is thus oriented in such a manner that the RGD cell signaling sequence is still solution accessible to mediate integrin binding events.

BI-WeP15 Conformational Studies of Human Salivary Histatin-5 Bound to Hydroxyapatite Surfaces and Lipid Bilayers, ***M. Cotten***, *J.L. Dindot, P.S. Stayton, G.P. Drobny,* University of Washington

Histatin-5 (hsn5) is a human salivary polypeptide found in the acquired enamel pellicle. The protein is histidine-rich and basic (DSHAKRHHGYKRKFHEKHHSHRGY), and possesses at least two important functions: control of HAP crystal growth and antimicrobial activity. Previous studies have characterized functionally important regions of the peptide sequence as well as some secondary conformation analysis in solution. Very little is known about specific hsn5/HAP and hsn5/lipid bilayer interactions and the conformation of the HAP-bound peptide. This knowledge is nevertheless necessary to better understand molecular recognition and structure-functions relationships. Our primary goals have been to characterize the conformation of hsn5 both free and bound to HAP crystals and lipid bilayers. Moreover, we have been interested in identifying interactions between the peptide and HAP by using solid state NMR techniques. Solid state NMR experiments that measure internuclear distances to sub-Angstrom accuracy have been performed to determine distances between two 13C carbonyl labels of adjacent amino acids and thereby constrain the conformation of the peptide bound to HAP. In addition, hisn5 with a 15N amide label has been incorporated into hydrated oriented lipid bilayers and used to determine the orientation of the peptide with respect to the bilayer.

BI-WeP16 Determination of Statherin N-Terminal Peptide Conformation on Hydroxyapatite Crystals, ***W.J. Shaw***, *J.R. Long, J.L. Dindot,* University of Washington*; A.A. Campbell,* Battelle, PNNL*; P.S. Stayton, G.P. Drobny,* University of Washington

The interactions between proteins and inorganic crystals play an important role in the development and growth of hard tissues such as bone and teeth. Although many of these proteins have been studied in the liquid state, there is little information describing molecular recognition at the protein-crystal interface. Here we have used 13C solid state NMR (SSNMR) techniques to investigate the conformation of an N-terminal peptide of salivary statherin both free and adsorbed on hydroxyapatite (HAP) crystals. The torsional angle phi was determined at three positions along the backbone of the N-terminal 15 amino acid peptide fragment (DpSpSEEKFLRRIGRFG) by measuring 13C-13C distances between carbonyls in adjacent amino acids using the Dipolar Recoupling with a Windowless Sequence (DRAWS) technique. The peptides adsorbed to the HAP surface have an average phi of -85 degrees at the N-terminus (SS), -60 degrees in the middle (FL) and -72 degrees at the C-terminus (IG). The SS position corresponds to an angle typically associated with random coil peptides. The FL and IG positions correlate with angles known to be alpha-helical. These angles are approximately the same in the lyopholized peptides implying that secondary structure content of the peptide is retained upon adsorption to the crystal surface.

BI-WeP17 Tethering Phospholipid Bilayers to Porous Substrates, Progress Towards Biosensor Development, ***S.D. Ogier***, *S.D. Evans, R.E. Miles,* Leeds University, UK

This poster concerns work currently being undertaken at Leeds University on the development of a biosensor based on ion channel conductivity. Our approach aims to span a lipid bilayer containing ligand gated ion channels across a hole micro-machined in a solid support. Electrodes either side of this hole allow the electrical properties of the bilayer to be monitored. The device employs a 0.1mm hole micro-machined in a solid support with a gold surface patterned around it. This enables us to modify the surface that the bilayer has to sit upon in order to create the conditions necessary for bilayer formation over the hole. Micro-machining of substrates has been used in a few cases to create architectures to suspend lipid bilayers and most of these use the solvent spreading method of Montal et al. or Langmuir Blodgett transfer. Although these techniques will be suitable for bilayer formation on the device, it is our aim to self-assemble the bilayer over the hole. This could be a very useful method of formation since it is a simple technique, however it does require the hole to have a similar diameter to the vesicle. The bilayer's electrical resistance is measured by applying a small (20mV) potential difference between two silver/silver chloride electrodes, one either side of the hole, and monitoring the current flowing between them. Although we have not yet achieved self-assembly over the holes, as they are too large, a bilayer formed using a solvent spreading method produced an electrical seal in the 10GOhm range.

BI-WeP18 Genetic Diagnostics Using SIMS Detection of Unlabeled DNA, ***T.J. Whitaker***, *K.F. Willey,* Atom Sciences, Inc.

We are developing a new DNA chip technology that uses secondary ion mass spectrometry (SIMS) to determine genetic diagnostic information from unlabeled DNA. Genosensor chips, or DNA chips, typically contain arrays of single-stranded oligodeoxynucleotide (ODN) probes. Each probe has a known sequence and is attached at a specific site on the chip. The sequence of an unknown target DNA is determined by binding single strands of the target to the probes. This binding, or hybridization, ideally will occur only where the target sequence is complimentary to the probe sequence. Evidence of the binding is generally obtained by detecting fluorescent or radioactive labels attached to the target DNA. The new technology uses peptide nucleic acids (PNA) instead of ODNs for the probes. PNA is a DNA analog in which the phosphate and deoxyribose are replaced with polyamides. PNA binds to DNA with the same A-T, G-C rules as DNA/DNA hybrids but has slightly higher melting temperatures, offering the potential for greater discrimination between single-base mismatches. As opposed to DNA, which contains one phosphate along the backbone at each base, PNA contains no phosphorus. We have exploited this difference by using a simple SIMS analysis to detect molecular fragments containing phosphorus. The existence of phosphorus positively identifies the hybridization of unlabeled DNA to PNA probes. This method and a

simplified, relatively inexpensive SIMS instrument are currently being developed under funding from the National Institutes of Health.@footnote 1@ @FootnoteText@ @footnote 1@This work is supported by the National Institutes of Health under contract 2 R44 HG01596-02. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

BI-WeP19 TOF-SIMS Analysis of the Candida Albicans Cell Surface, ***H. Shi***, *B.J. Tyler,* Montana State University

The most common cause of failure for medical devices is infection, and the pathogenic yeast Candida albicans is the third leading cause of these infections. Adhesion is the first step in establishing an infection, the initial adhesion of microorganisms to synthetic polymer surfaces involves physicochemical interactions between molecules present at the polymer surface and those present on the cell surface. These interactions are not well defined, and insight into this area could lead to better material construction to effectively control the infection. The objective of this thesis is to study the initial adhesion event of C. albicans grown in glucose-based medium and galactose-based medium to Fluorinated Ethylene Propylene(FEP) and use Secondary Ion Mass Spectrometry(SIMS) to study the surface structural differences between these two cell surfaces with an attempt to correlate surface functionalities to the adhesion results. A freeze-drier with ultimate vacuum less than 1.00x10-9torr was constructed "in house" to freeze-dry cells for SIMS analysis. A filtration method was used to prepare a smooth layer of cells to be freeze-dried. Scanning Electron Microscopy showed that the freeze-dried cells appeared intact. High resolution SIMS spectra were obtained from these freeze-dried cell surfaces and Linear Discriminant Function Analysis combined with Principle Components Analysis were used to analyze the SIMS spectra. The results showed that the surfaces of glucose-grown cells contained more hydrophobic amino acid residues relative to those of galactose-grown cells. These hydrophobic amino acid residues probably promoted the adherence of glucose-grown cells to FEP surface. The results obtained in this study suggest that hydrophobic interactions are important in the initial attachment of C. albicans to FEP surface. The SIMS spectra presented in this study were the first report of the freeze-dried C. albicans.

Biomaterial Interfaces Group
Room 613/614 - Session BI-ThM

Biomineralization

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

8:20am **BI-ThM1 Nucleation and Growth of Calcium Phosphate on Self-Assembled Monolayers**, ***C.C. Chusuei,*** Texas A&M University; *B.J. Tarasevich,* Pacific Northwest National Laboratory; *D.L. Allara,* Pennsylvania State University; *M.J. Van Stipdonk, E.A. Schweikert, D.W. Goodman,* Texas A&M University

Calcium phosphate (CP) was adsorbed onto 16-carbon chain length self-assembled monolayers (SAMs) with various terminated functional groups from solution simulating ionic conditions found in blood plasma at the bone growth region at various exposure times to observe the onset of nucleation growth. X-ray photoelectron spectroscopy (XPS) was used to quantitate nucleation and growth of CP on the surface and compared with ellipsometric measurements. Secondary ion mass spectrometry (SIMS) was used for speciation, monitoring transformation from amorphous phases to hydroxyapatite and comparing them to standardized CP powders.@footnote 1@ Accelerated nucleation and growth of CP on anionically charged SAMs terminal functional groups, -COOH and -SO@sub 4@H, was readily observed at 310 K (consistent with physiological conditions) relative to room temperature (298 K). No growth was observed on the -CH@sub 3@ terminated SAMs. @FootnoteText@ @footnote 1@ Chusuei, C. C.; Van Stipdonk, M. J.; Justes, D. R.; Schweikert, E. A.; Goodman, D. W. Anal. Chem. 1999, 71, 149-153.

8:40am **BI-ThM2 Surface Modification of a Model Biomaterial by UV-Laser and/or Electron Beam Irradiation@footnote 1@**, ***M.L. Dawes,*** Washington State University; *Y. Kawaguchi,* Chugoku National Industrial Research Institute, Japan; *S.C. Langford, J.T. Dickinson,* Washington State University

Single crystal brushite (CaHPO@sub 4@@super .@2H@sub 2@O) is a model hydrated phosphate for studies of surface modification, etching, and biocompatible film growth by laser ablation. In this study we show that significant chemical and morphological changes are produced on such crystal surfaces by irradiation with electron and UV-laser beams. These changes are due to both photoelectronic and thermal effects, principally involving the anion, and are associated with high densities of point defects. We compare the spectroscopic and morphological changes generated by laser and electron beam irradiation as well as by thermal treatment in vacuum. All three treatments dehydrate the surface material; the resulting material forms subsurface, micron-sized platelets which can be exposed by spontaneous fracture of the surface layer. Spectroscopic evidence for reduced forms of phosphorus (primarily pyrophosphate but also elemental phosphorous) are observed on the treated material. Mass spectroscopy of laser-induced emissions from treated material show significant O@sub 2@ and PO@sub x@ emissions, consistent with this reduction. @FootnoteText@ @footnote 1@ This work is supported by the Department of Energy (DE-FG03-98ER14864) and the National Science Foundation (CMS-98-00230).

9:00am **BI-ThM3 Synthesis and Surface Characterization of Peptide-Modified Interpenetrating Polymer Networks that Control Biomineralization**, ***K.E. Healy,*** *T. Barber,* Northwestern University; *D.G. Castner, S.L. Golledge,* University of Washington **INVITED**

A major limitation in the performance of materials used in the medical device and pharmaceutical industries is that they lack the ability to integrate with biological systems through either a molecular or cellular pathway. We have designed and synthesized interfacial interpenetrating polymer networks (IPNs) that resist non-specific protein adsorption, and can be modified to tether bioactive groups such as peptides that mimic cell binding domains found on ECM molecules. An IPN was created by sequential photoinitiated synthesis of a thin layer of poly(acrylamide) [P(AAm)] followed by a secondary photoinitiation step using poly(ethylene glycol) [PEG] based monomers to create the network. Tethering of peptides was achieved by photoinitiated synthesis of PEG-monomethyl ether monomethacrylate, acrylic acid (AAc) and N,N-methylene-bis-acrylamide into the P(AAm) layer. A spacer of bisamino PEG (3400 MW) was then bonded to the AAc through a carbodiimide reaction. As a specific example of coupling bioactive molecules to the surface, peptides from the cell binding domain [CGGNGEPRGDTYRAY] and heparin binding domain [FHRRIKA] of bone sialoprotein were tethered to the remaining free PEG amine moiety via a sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate cross linker. Surfaces were characterized by contact angle measurements, spectroscopic ellipsometry, and X-ray photoelectron spectroscopy. The surface characterization confirmed the formation of the IPN and subsequent immobilization of the peptide. These surfaces resisted protein deposition and neither supported cell attachment nor growth without immobilization of the RGD-based biomimetic peptide from bone sialoprotein. Molecular modification of the non-adhesive IPN using a RGD-containing peptide led to rapid bone cell attachment independent of the presence of serum proteins, and subsequently to normal cell proliferation and normal phenotypic expression (e.g., synthesis of mineralized matrix).

9:40am **BI-ThM5 Osteoblast Behavior on Surfaces with Varied RGD Peptide Surface Concentrations Prepared Using Gold-Thiol Self-Assembly**, ***G.D. Moodie,*** *D.M. Ferris, R.F. Henn, N.J. Wimmer,* Brown University; *R.F. Valentini,* Brown University / Rhode Island Hospital

In this work we evaluate the response of osteoblasts to changing concentrations of the integrin-binding RGD peptide immobilized on gold-coated surfaces. Surfaces were prepared by evaporating 100 Å of titanium onto glass cover slips followed by 800 Å of gold. The peptide Arg-Gly-Asp-Cys (RGDC) and the diluent Cys were bound to the gold through the Cys thiol. 1:0, 1:1, 1:10, 1:100, 1:1000, 1:10000, and 0:1 RGDC:Cys solutions were tested. XPS and SIMS verified peptide immobilization. Osteoblasts isolated from 6-day old rat calvaria were plated at a density of 10,000 cells/cm@super 2@ for one hour and then fixed. Co-localization of actin and vinculin indicates the presence of integrin-based focal adhesions. Vinculin was stained with mouse anti-human vinculin IgG and a rhodamine conjugated secondary antibody. Actin staining was done with FITC / phalloidin. Peptide stability was first assessed by aging uncoated, RGDC coated, and fibronectin coated substrates for 3, 9, 14, and 28 days in serum-free media. Co-localizations were observed on 85-90% of cells on RGDC substrates that had been aged for 3 to 28 days. In contrast, co-localization on fibronectin coated surfaces showed a steady decline with aged specimens and was at the level of plain gold by day 28 (about 30%).The percent of cells showing co-localizations, the number of co-localizations per cell, and cell area all decreased as peptide concentration decreased and were statistically different from 100% RGDC at and below the 1:100 dilution. This study shows that RGDC binds to gold surfaces and influences osteoblast response in a dose-dependent fashion.

10:00am **BI-ThM6 Molecular Recognition at the Protein-Biomineral Interface**, ***J.R. Long,*** *W.J. Shaw, G.P. Drobny, P.S. Stayton, P. Bower,* University of Washington

Biological organisms exhibit sophisticated crystal engineering capabilities that underlie the remarkable material properties of mineralized tissues such as bone and nacre. While nature's biomineralization processes are a complex blend of finely controlled nucleation and growth events that are not currently well understood, it is known that organisms produce acidic proteins which play a key directoral role in controlling biological crystal growth. We have taken a systematic approach with model proteins and biological proteins and peptides to elucidate how small, acidic proteins interact with biological crystals and control their growth rates. Solid-state NMR results investigating protein conformation and orientation on HAP surfaces will be reported.

10:20am **BI-ThM7 Kinetics and Interfacial Energy Studies of Biomineralization**, ***G.H. Nancollas,*** *W. Wu,* State University of New York, Buffalo **INVITED**

The ability of surfaces to nucleate minerals such as the calcium phosphates is important in a wide range of biological events. The kinetics of crystallization and dissolution of the mineral surfaces has received considerable attention from the point of view of parameters such as solution composition, ionic strength, pH, temperature, and solid surface characteristics. However, a factor which is usually ignored in discussions of such induced crystallization reactions is the surface free energy of the nucleus/substratum interface. The Constant Composition method is especially useful for investigating the mechanisms of these reactions and surface free energies, measured using thin layer wicking methods can be used to corroborate crystal growth and dissolution mechanisms determined from kinetics experiments. Kinetic studies have been made using calcium phosphate phases such as dicalcium phosphate dihydrate (DCPD), octacalcium phosphate (OCP), hydroxyapatite (HAP), and fluorapatite (FAP). The much smaller interfacial tensions of OCP and DCPD in contact with water as compared with those of HAP and FAP support the widely held suggestion that the former phases are precursors in HAP and FAP biomineralization. On substrata consisting of minerals, polymers or typical implant materials such as the titanium oxides, the ability of the

surfaces to nucleate calcium phosphate minerals is closely related to the magnitude of the interfacial energies.

11:00am **BI-ThM9 Incorporation of Dye Molecules into Calcium Oxalate Host Crystals**, ***L.A. Touryan***, *R.W. Gurney,* University of Washington*; M.J. Lochhead,* University of New Hampshire*; B. Kahr, V. Vogel,* University of Washington

Biological systems direct inorganic mineral synthesis and subsequent composite growth via molecular interactions between macromolecules and mineral phases. However, describing the relationship between the organic and inorganic molecules and their interactive functions at the molecular level remains difficult. Exactly how additives orient within host lattices is not known, as biomineral crystals accommodate their presence but they do not give rise to sufficient intensity in x-ray diffraction studies. Nevertheless, it is this integration of large biomolecules into much smaller unit cells that profoundly changes the materials properties of biominerals and make their synthetic recreation desirable for novel material design and the enhanced biocompatibility of biomedical implants. Here we discuss the use of sensitive optical techniques, in conjunction with modeling, to determine the spatial orientation of organic additives within the host lattice of a calcium biomineral. We have found that common aryl-carbonium dye molecules such as eosin and fluorescein incorporate into the lattice structure of calcium oxalate, the primary biomineral of kidney stones. These dyes tend to incorporate along the fastest growing crystal planes, and can be detected through fluorescence microspectroscopy. We have measured the intensity of polarized fluorescence on two well-developed crystal faces, calculated dichroic ratios, and used the data to model the direct orientation of the transition dipole moments of organic dyes that incorporate within the inorganic host lattice.

11:20am **BI-ThM10 Molecular Orientation in Artificial Joint Polymers: Characterizing the Precursors of Wear with Soft X-ray Absorption**, ***D.A. Fischer,*** National Institute of Standards and Technology*; S. Sambasivan,* Brookhaven National Laboratory*; M. Shen,* University of Maryland, College Park*; S. Hsu,* National Institute of Standards and Technology

Over half a million patients receive artificial joint replacements annually and practically all the replacements consist of a sliding pair represented by a polymer (ultra-high molecular weight polyethylene -UHMWPE) and a hard counterface (metal or ceramic). For the past 30 years UHMWPE has remained the dominant polymer in artificial joints due to its outstanding wear resistance properties. It has been recognized that wear of UHMWPE contributes to the loosening of the implants and is the main cause for the failure of long-term implants. Hence there is an urgent need to understand the mechanism and the surface morphology leading to wear and failure of the artificial joint. Molecular orientation in biomaterials is thought to be critical in characterizing the precursors of wear and the production of debris during the wear process. Current methods of inferring or deducing orientation are not accurate and often rely on staining and cutting specimens. In this study we use the electric field polarization dependence of soft x-ray absorption to directly determine molecular orientation in UHMWPE and evaluate the utility of this technique for evaluating artificial joint materials. We have measured the change in molecular orientation of ultra high molecular weight polyethylene (UHMWPE) samples subjected to various wear motions and duration. Two motions were used: a unidirectional and a cross-shear (motion to form figure-eight) motion. The observed orientations of the UHMWPE molecular chains using soft x-ray absorption are discussed and contrasted with the current understanding of the wear process in UHMWPE.

11:40am **BI-ThM11 Peptide Functionalized Titanium Alloy Surfaces for Orthopedic and Dental Materials**, ***F.A. Akin***, *L. Hanley,* University of Illinois, Chicago*; H. Zreiqat, C.R. Howlett,* University of New South Wales, Australia

Surface modification to a biomaterial may improve long term survival of prosthetic devices. The modulation of bone behavior was examined by surface chemical modification of titanium alloy (Ti-6Al-4V) using peptides. RGDSC (arginine-glycine-aspartate-serine-cysteine) was covalently bound to the Ti-6Al-4V surface by 3-aminopropyltriethoxysilane. Surface characterization of amine-, cysteine-, and RGDSC-terminated Ti-6Al-4V was determined using x-ray photoelectron spectroscopy, roughness assesment, and scanning electron microscopy. All elemental peaks as well as the valence band are employed in the x-ray photoelectron spectral analysis of RGDSC on Ti6Al4V. The S(2p) peak was used to determine the atomic percentage of S on the surface, providing information on the peptide surface density. The valence band of the XPS also showed significant differences between the three surfaces. The attachment and proliferation of human bone-derived cell (HBDC) to the amine-, cysteine-, and RGDSC-terminated Ti-6Al-4V were examined using colorimetric and immunohistochemical assays.

Surface Science Division
Room 606 - Session SS1+AS+BI-ThM

Self-Assembled Monolayers

Moderator: N.D. Shinn, Sandia National Laboratories

8:20am **SS1+AS+BI-ThM1 Two-Dimensional Phase Transitions in Amphiphile Monolayers**, ***G.E. Poirier,*** National Institute of Standards and Technology

Recently scientists have explored methods of constructing complex chemically patterned surfaces with the goal of making novel biosensors or of realizing lab-on-a-chip technology. In order to design patterned monolayers it is important to understand how these molecules behave in two dimensions, what is the molecular packing of the surface phases and which phases coexist in thermodynamic equilibrium. The goal of this presentation is to describe the two-dimensional structural phases of decanethiol monolayers. Our studies were conducted using gas-phase transport of decanethiol onto clean Au(111) in an ultrahigh vacuum scanning tunneling microscope. With increasing surface coverage, the monolayer sequentially adopts six discrete structural phases. At low surface coverage, decanethiol exists as a lattice gas. With increasing coverage the molecules sequentially condense into islands of three discrete commensurate crystalline lattices, each characterized by alignment of the molecular axes with the surface plane, but with discretely increasing degrees of out-of-plane interdigitation. Above saturation coverage of the densest surface-aligned phase, the monolayer undergoes an edge-mediated melting transition. The c(3x2/3) phase, characterized by alignment of the molecular axes close to the surface normal, nucleates and grows from this liquid. These studies provided a detailed, real-space picture of the coverage-dependent phases and phase transitions of alkanethiol molecular monolayers on Au(111).

8:40am **SS1+AS+BI-ThM2 Surface Phase Transitions of Asymmetric Dialkyl Disulfide Self-Assembled Monolayers**, ***M. Hara***, *K. Kamei, T. Araki, K. Fujita, W. Knoll,* Frontier Research Program, RIKEN, Japan

Adsorption and desorption processes of asymmetric dialkyl disulfide (hexyl octadecyl disulfide: C6-SS-C18) self-assembled monolayers (SAMs) on Au(111) have been investigated by surface plasmon resonance (SPR), mass spectroscopy (MS), thermal desorption spectroscopy (TDS), and scanning tunneling microscopy (STM). Formation of C6-SS-C6 and C18-SS-C18 dimer species in the monolayer and also in the solution through dimerization and exchange processes has been confirmed during the monolayer growth. In the TDS spectra for shorter immersion SAMs, C6 monomer species showed the strong peaks, and gradually C6-SS-C6 dimer and C18 monomer peaks become stronger for longer immersion. Phase-separated domains of pure C6 and pure C18 striped phases have been visualized in the initial growth stage by STM and the area of the C6 domain was larger than that of C18 one. These results suggest that S-S bonds were cleaved when disulfides adsorbed on Au surface followed by the surface diffusion to form phase separation before standing-up SAM formation. After the C6-rich SAM growth, molecules are dimerized and then exchange process starts from the weakly bound C6-SS-C6 dimers toward C18-rich SAM. SPR kinetics studies also showed the double exponential growth which is different from usual Langmuir adsorption isotherm. Following those results, we propose more detailed surface phase transition model of alkanethiol SAM growth through the dimerization and the exchange processes.

9:00am **SS1+AS+BI-ThM3 Structure, Bonding and Reactivity of Self-assembled Monolayers**, ***G.J. Leggett***, *B.D. Beake, N.D. Brewer,* UMIST, UK*; E. Cooper,* Glaxo-Wellcome, UK*; D.A. Hutt,* University of Loughborough, UK

Two approaches to the characterisation of the nature of the sulfur bonding environment in self-assembled monolayers (SAMs) have been explored: sulfur K-edge surface extended X-ray absorption fine structure (SEXAFS) and static secondary ion mass spectrometry (SIMS). S K-edge SEXAFS has proved highly effective for SAMs on Ag and has confirmed that the sulfur adsorbs with threefold coordination during both solution-phase adsorption onto polycrystalline Ag and gas-phase adsorption onto Ag(111) single crystal surfaces. There is no change in the S adsorption site with coverage. Static SIMS has yielded powerful insights into the effect of electron beam bombardment on SAM structure. A complete loss of all gold-molecular fragments from the spectrum is observed after small doses of electrons, indicating a rapid alteration of the sulfur bonding environment, due either

to S-C bond scission or, more likely, to oxidation of thiolates to disulfides. Changes in Au-S bonding during the formation of the low coverage phase of butanethiol on Au have also been studied by static SIMS. Photo- and air-oxidation rates are influenced by the adsorbate alkyl chain length; rates of both processes decrease with increasing chain length due to increasing SAM ordering. The nature of the terminal group also affects rates of oxidation, and has a profound influence on the stability of the SAM. Hydrogen bonding between neighbouring terminal groups leads to significant stabilisation. Friction coefficients may be measured for SAMs using friction force microscopy. These decrease with increasing alkyl chain length, and are higher for adsorbates with polar terminal groups. Oxidation of methyl terminated SAMs leads to an increase in the coefficient of friction, interpreted in terms of the collapse of film order following head group oxidation. The rate of increase in the coefficient of friction is faster for short chain SAMs than long-chain SAMs, in agreement with the findings of static SIMS studies.

9:20am **SS1+AS+BI-ThM4 Structure of Partially Fluorinated n-alkanethiols on Polycrystalline Gold and Silver Surfaces**, ***S. Frey***, Universität Heidelberg, Germany; *K. Tamada,* National Institute of Materials and Chemical Research, Japan; *K. Heister, M. Zharnikov, M. Grunze,* Universität Heidelberg, Germany

The physical and chemical properties of self-assembled monolayers (SAM) of alkanethiols (AT) are noticeably affected by partial fluorination of the hydrocarbon chain. We have studied the structure of SAMs formed from partially fluorinated alkanethiols (PFAT) CF@sub 3@(CF@sub 2@)@sub 9@(CH@sub 2@)@sub n@SH with different hydrocarbon chain length (n = 2, 11 and 17) on Au and Ag coated silicon wafers. The objectives of our experiments were (i) to find out whether PFAT form ordered and densely packed SAMs on these substrates, (ii) to determine the individual conformation and orientation of the hydro- and fluorocarbon parts in the molecules, and (iii) to clarify the influence of the headgroup-metal bond (RS-Au or RS-Ag) on the film structure. The PFAT films were characterized by atomic force microscopy, infrared reflection absorption spectroscopy, X-ray photoelectron spectroscopy, and angle resolved near edge X-ray absorption fine structure spectroscopy. PFATs were found to form highly ordered and densely packed SAMs on polycrystalline Au and Ag. The hydrocarbon and fluorocarbon chains of the adsorbed PFATs retain the expected planar zigzag and helical conformation of the bulk materials, respectively. The fluorocarbon chains, which are oriented almost perpendicular to the substrates for CF@sub 3@(CF@sub 2@)@sub 9@(CH@sub 2@)@sub 2@SH, assume a more tilted orientation in PFAT SAMs with longer hydrocarbon moieties. As found for the AT on Ag and Au, the hydrocarbon part in the PFAT films exhibits smaller tilt and twist angles on Ag as compared to the Au substrates. Considering the reduced van der Waals interaction between the hydrocarbon chains in PFAT as compared to neat AT SAMs (due to the sterical constraints provided by the fluorocarbon chains), the differences in tilt and twist angle appears to be associated with the different character of the headgroup-substrate bonding on Au and Ag. This work has been supported by the German Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie through grant No. 05 SF8VHA 1.

9:40am **SS1+AS+BI-ThM5 Controlling the Placement of Molecules in the Self Assembly and Directed Assembly of Organic Monolayers**, ***P.S. Weiss***, *D.L. Allara, L.A. Bumm, J.J. Jackiw,* The Pennsylvania State University **INVITED**

We manipulate and measure the structures of monolayer films in order to tune their properties. This is accomplished by controlling the defect type and density in the films. We then process the films to insert single molecules, to insert bundles of molecules, or to graft new molecular terraces onto existing domains by using these defects to advantage. The inserted molecules can serve as the anchor points for polymerization; this allows us to choose to produce single polymer dots or isolated polymer brushes. We connect our scanning tunneling microscopy measurements to electron transfer phenomena which are ubiquitous in such areas as biochemistry and electrochemistry by separating the transconductance into components arising from transport through the molecule vs. the tunneling gap outside the film. We show how these components can be measured independently. We prepare films predicted to have many equivalent defect sites so as to provide identical matrix isolation environments for single molecular wire candidates. We also prepare films with well defined interfaces between separated components so that insertion, deposition, or reaction can be directed to these molecularly sharp boundaries.

10:20am **SS1+AS+BI-ThM7 Protein Adsorption to Model Surfaces: Probe Adhesion Between Fibrinogen and Patterned SAMs**, ***L.F. Pardo***, *T.B. Boland,* Clemson University

Interactions between blood and an artificial surface induce a rapid, thrombogenic response believed to be mediated by protein adsorption. In this study, atomic force microscopy (AFM) was used to measure directly non-specific forces between proteins and functionalized surfaces. A protein-modified AFM cantilever tip was used to probe of interactions between a model substrate and a single protein. Model surfaces consisted of both simple and microcontact-printed, -OH, -COOH, and -CH3 terminated self-assembled monolayers (SAMs) of alkanethiols on gold. Fibrinogen was used as the model protein due to its significance in biomaterial-mediated inflammatory responses. It was tethered to the probe tip using a synthetic polypeptide (polyserine). Various approaches were taken to link polyserine to a self-assembled monolayer on a gold substrate. Ellipsometric and vibrational spectroscopic measurements indicated that successive carbodiimide activation of a carboxyl-terminated alkanethiol and polyserine allowed for the successful formation of a polyserine-tethered fibrinogen film. The SAMs, polyserine and fibrinogen films were characterized by ellipsometry and contact angle measurements. Both AFM images and measurements will be presented and discussed. An understanding of how fibrinogen interacts under model conditions will give insight into more complicated real systems.

10:40am **SS1+AS+BI-ThM8 Modification of Self-Assembled Monolayers by X-ray, Electron and Thermal Treatments**, ***H. Fairbrother***, *A. Wagner, K. Han,* Johns Hopkins University

Polymer surface modification strategies are widely used to modify interfacial characteristics, including permeability, wettability, adhesion, friction, wear and biocompatibilty. To better understand the detailed nature of these processes thiol-based self-assembled monolayers (SAMs) adsorbed on Au substrates have been used as models for the polymeric interface. In the case of X-ray modification results from X-ray Photoelectron Spectroscopy on fluorinated SAMs show that the degree of cross-linking can be directly correlated with the appearance of CF groups in the organic film. Additional information from Reflection Absorption Spectroscopy and Atomic Force Microscopy on the impact of X-ray, electron, and thermal modification treatments will also be presented, enabling a detailed picture of the chemical and physical modifications that occur during surface treatments to be constructed.

11:00am **SS1+AS+BI-ThM9 Electron-induced Damage in Thio-functionalized Alkanethiol Monolayers**, ***K. Heister***, *W. Geyer, S. Frey,* Universität Heidelberg, Germany; *A. Ulman,* Polytechnic University; *A. Gölzhäuser, M. Zharnikov,* Universität Heidelberg, Germany

Potential technological applications of self-assembled monolayers (SAM) of alkanethiols (AT) as lithographic resist require an increased sensitivity of these systems toward ions, X-ray photon or electron irradiation. This effect can be achieved by incorporation of specific molecular groups associated with comparatively weak bonds, such as C-S bond provided by sulfide -S- and sulfone SO@sub 2@- moieties, in the aliphatic chains. We have investigated the damage induced by low-energy electrons in SAMs formed from C@sub 6@H@sub 13@SC@sub 11@H@sub 22@SH (I), C@sub 6@H@sub 13@SO@sub 2@C@sub 11@H@sub 22@SH (II), C@sub 11@H@sub 23@SO@sub 2@C@sub 6@H@sub 12@SH (III), and C@sub 11@H@sub 23@SO@sub 2@C@sub 6@H@sub 4@OC@sub 5@H@sub 10@SH (IV) on polycrystalline Au substrates using angle-resolved near edge X-ray absorption fine structure spectroscopy and X-ray photoelectron spectroscopy. Similar to AT SAMs, an irradiation-induced disordering and a dehydrogenation of the pristine films I - IV as well as the desorption of molecule fragments and the transformation of thiolate moieties in a new irradiation-induced sulfur species were observed. The extent of the irradiation-induced events is, however, affected by the introduction of the sulfide and sulfone moieties. An increased extent of the irradiation-induced desorption processes as compared to AT SAMs was found in I and II, which implies that bond scission events predominately occur in the outermost part of AT SAM. Controversially, an additional dipolar interchain interaction provided by the sulfonic entities has a stabilizing influence on the lower part of SAMs II-IV leading to the conservation of the pristine thiolate species responsible for anchoring of the alkyl chains to the substrate. Considering these controversial effects provided by the sulfonic groups the incorporation of sulfide moiety seems to be a better choice to improve the sensitivity of AT SAMs toward electron irradiation.

11:20am **SS1+AS+BI-ThM10 Sensitivity of Alkanethiol Self-assembled Monolayers toward Low-energy Electron Irradiation***, **M. Zharnikov**, S. Frey, K. Heister, M. Grunze,* Universität Heidelberg, Germany

Potential technological applications of self-assembled monolayers (SAM) as lithographic resist involve their exposure to ions, X-ray photons or electrons. We have investigated the damage induced by low-energy electrons in SAMs of dodecanethiolate (DDT), octadecanethiolate (ODT) and perdeuterated eicosanethiolate (PDET) on gold and of ODT on silver using x-ray photoelectron spectroscopy and angle resolved near edge x-ray absorption fine structure spectroscopy. ODT/Au was taken as a reference system, whereas the other SAMs differed from ODT/Au in the length (DDT/Au) and the isotopic composition (PDET/Au) of the alkyl chains and the strength and character of the thiolate-metal bond (ODT/Ag). All systems studied were found to exhibit a qualitatively similar behavior with respect to low-energy electron irradiation. Both the alkyl chains and the S-Au interface are affected simultaneously through the electron-induced dissociation of C-H, C-C, C-S, and metal-thiolate bonds. The most noticeable processes are the loss of the orientational and conformational order, partial dehydrogenation with C=C double bond formation, desorption of film fragments, decrease of thiolate species, and the appearance of new sulfur species. The cross sections for the individual irradiation-induced processes have been determined. The reactions of the alkyl matrix and the S-metal interface to electron irradiation are essentially independent. The extent and rate of thiolate species reduction and new sulfur species formation are mainly determined by the strength and character of the thiolate-metal bond (Au vs. Ag). At the same time, an extent of irradiation-induced desorption of sulfur-containing fragments depends on the alkyl chain length. Only a slight isotopic variation in the irradiation-induced dehydrogenated process was observed.

Surface Science Division
Room 613/614 - Session SS3+BI-ThA

Biological Surface Science

Moderator: B. Kasemo, Chalmers Univ. of Tech. and Göteborg Univ.

2:40pm **SS3+BI-ThA3 Thia(Ethylene Oxide) Alkanes: Tuning the Structure of a Supported Biomimetic Membrane via Spacer Length and Packing Density**, *D.J. Vanderah, C.W. Meuse, T. Petralli-Mallow,* ***A.L. Plant,*** National Institute of Standards and Technology **INVITED**

Interest in reconstituting transmembrane proteins in supported cell membrane mimics of phospholipid/alkanethiol hybrid bilayers has led to development of novel tethering molecules. In an attempt to introduce a disordered, hydrophilic region at the proximal side of the supported lipid bilayer, ethylene oxide moieties have been used as spacers between the sulfur and the alkane chain of alkanethiols. We have observed by infrared spectroscopy, neutron reflectivity, and electrochemistry that such ethyleneoxide moieties are not necessarily hydrated or disordered. The ethylene oxide of thiahexa(ethylene oxide) alkanes (HS(EO)@sub 6@-alkanes) form a highly ordered arrangement of 7/2 helices when allowed to self-assemble at gold from an ethanolic solution. This highly ordered conformation is apparently not driven by order in the alkane chains, since the helical structure occurs in both HS(EO)@sub 6@-decane and HS(EO)6-octadecane. The conformation is, however, determined in part by molecular density. Infrared analysis of mixed monolayers of HS(EO)@sub 6@-alkanes and phospholipids transferred from the air-water interface indicates that at low packing densities the EO region is disordered, but it assumes the helical structure at higher packing densities. Infrared spectroscopy and sum frequency generation suggest that the conformation of the EO region may be controlled by its length. For a series of self-assembled monolayers of HS(EO)@sub n@-decanes, the EO segment is an extended all-trains chain when n=4, a 7/2 helix when n=5-7, and a less ordered conformer when n=8. This effect of spacer length and packing may provide a means of tuning the molecular order of biomimetic matrices.

3:20pm **SS3+BI-ThA5 Quantitative Analyses of Biological Interactions using Surface-Biofunctionalized Surface Plasmon Resonance Devices**, ***C.T. Campbell,*** *L.S. Jung, J. Shumaker-Parry, K.E. Nelson, P.S. Stayton, M.S. Boeckl, M.H. Gelb, S.S. Yee, T. Sasaki, R. Aebersold,* University of Washington **INVITED**

The adsorption of molecules from liquid solutions onto solid surfaces can be monitored with high sensitivity and fast time response by following changes in the angle or wavelength at which the surface plasmon resonance (SPR) of a thin metal film is optically excited. Simple methods convert these measured changes into adsorbate concentrations. We report here the adsorption and desorption kinetics and equilibrium coverages of a variety of species on well-characterized surfaces as determined by SPR techniques. When the diffusion constant of the adsorbing species is known in the liquid phase, the intrinsic rate constants can be determined from the kinetic results. A new method will be described for converting intrinsic adsorption rate constants into sticking probabilities (i.e., the probability that adsorption occurs, given a collision of the molecule with the surface). Several applications of gold-thin-film SPR sensors in quantifying biological interactions will be described. A gold surface containing a few biotin headgroups in a self assembled alkylthiol monolayer of mainly oligo(ethylene glycol) headgroups has been used to assess the effects of protein mutations on the strength of biotin-streptavidin binding. With wild-type streptavidin, the free biotin sites in the resulting streptavidin monolayer have been used as strong linker sites for further attachment of intact, biotinylated lipid vesicles and biotinylated, double-stranded oligonucleotides to the surface. These complex biological films then provide a surface template that can be used to probe the kinetics and equilibrium binding constants for: (1) peripheral membrane proteins binding to vesicle walls, and (2) the binding of DNA-binding proteins to select oligonucleotide sequences.

4:00pm **SS3+BI-ThA7 Adsorption and Reactions in Enzymes and on Surfaces: Similarities And Differences**, *A. Logadottir, T.H. Rod,* Technical University of Denmark*;* ***J.K. Nørskov,*** Technical University of Denmark, Denmark **INVITED**

There are a number of cases where surfaces and biomolecules adsorb the same molecules and catalyze the same reactions. This makes it possible to make comparisons and to see if the concepts of surface science can be transferred to biomolecules. As a specific example, the adsorption of N@sub 2@ and the ammonia synthesis reaction on metal surfaces and in the enzyme nitrogenase will be compared in detail. The comparisons are largely based on density functional calculations which provide a detailed description of the extensive database of experiments for the nitrogen activation process on Fe and Ru surfaces, and which can be used to give the first hint of a mechanism in the enzyme process.

4:40pm **SS3+BI-ThA9 The Role of Hydrogen Bonding in Chemisorbed Aminoacids**, ***N.V. Richardson,*** University of St Andrews, UK **INVITED**

Glycine and its derivatives such as phenyl glycine and alanine form well-ordered monolayers of the corresponding anion on Cu(110). The unit cells reflect both the strong adsorbate-substrate interaction and the hydrogen bonding interactions between adsorbed species. In the case of the chiral amino acids studied, this leads to ordered domains of the two isomers which are distinguishable. Glycine undergoes several orientational changes during the build up of the ordered monolayer and is then able to form a stable bilayer. Such a bilayer is not formed in the case of phenyl glycine or alanine demonstrating the importance of optimal hydrogen bonding in stabilising the bilayer. Adsorption of water on the glycine covered copper surface drives a re-orientation of the molecule which we also relate to the importance of hydrogen bonding between the co-adsorbed species.

Biomaterial Interfaces Group
Room 613/614 - Session BI-FrM

Interface, Properties, and Modification

Moderator: B.D. Ratner, University of Washington

8:20am **BI-FrM1 Topographical Polymorphism of a Phospholipid Monolayer**, ***W.R. Schief***, *L.A. Touryan,* University of Washington; *S.B. Hall,* Oregon Health Sciences University; *V. Vogel,* University of Washington

Light scattering microscopy reveals previously undetected topographical complexity in lipid monolayers at the air/water interface. At a surface pressure (@pi@) of @pi@ = 13 mN/m at room temperature, following completion of the liquid-expanded (LE) -> liqu id-condensed (LC) transition, the LC phase of Dipalmitoylphosphatidylcholine (DPPC) develops corrugations within a region covering half the monolayer and surrounding flat, chiral-shaped domains. The scattered intensities of the domains and the surrounding region are analyzed in light of capillary wave theory. With compression over @pi@ = 20 mN/m, the corrugated region becomes decorated with nanoparticles through a reversible budding process. Beyond a threshold of @pi@ = 60 mN/m, the budding accelerates. A tomic force microscopy (AFM) on samples transferred to mica confirms the presence of multibilayer discs of diameter 15 - 150 nm. These findings provide new information on potential surface mechanisms of respiration, since a monolayer enriched in DPPC is widely thought to coat the lung.

8:40am **BI-FrM2 Plasma Co-polymer Surfaces for the Controlled Adsorption of Common Proteins**, ***J.D. Whittle***, *R.D. Short, C.W.I. Douglas,* University of Sheffield, UK; *J. Davies,* Johnson and Johnson Orthoclinical Diagnostics, UK

The topic of protein adsorption is of key interest in biomaterials science, since it is generally believed that subsequent surface reactions are guided by the composition of adsorbed proteins. This is especially pertinent in immunodiagnostics, where nonspecific and poorly characterised protein binding may lead to false positives and poor signal-noise ratios. We are interested in controlling the passive adsorption of several common proteins from single solutions through the molecular engineering of surfaces by means of plasma. We utilise continuous wave rf plasma copolymerisation to allow us to synthesise ultra-thin plasma polymer (PP) films of controllable surface chemistry, from various starting 'monomers'. The plasma polymers are analysed by XPS to allow us to estimate the proportion of different functional groups present in the deposited surface. In this experiment we examine surfaces deposited from plasmas of allyl alcohol and acrylic acid. A range of surface functionalities was produced by copolymerising the functional monomer with 1,7-octadiene. Protein adsorption was estimated by an Enzyme Immunoassay (EIA) after exposing the surfaces to single solutions of human albumin, fibrinogen, vitronectin and IgG overnight at a temperature of 37°C and a pH of 7.0. Results show that the amount of protein adsorbed depends not only upon the protein being investigated, but also the characteristics of the polymer surface, with a clear correlation between functional group concentration and the adsorption of fibrinogen, vitronectin and IgG. The adsorption of albumin is not affected by the funtionality of the surface, however this may reflect a limitation in the technique. SPR measurements suggest that even at low concentrations albumin can form a multilayer on these plasma deposited surfaces, which would lead to poor sensitivity of the assay.

9:00am **BI-FrM3 Reversible Immobilization of a Thermally-Responsive Fusion Protein on a Hydrophobic Surface**, ***W. Frey***, *N.A. Hattangadi, D.E. Meyer, A. Chilkoti,* Duke University

Elastin-like polypeptides (ELPs), which are composed of repeats of the Val-Pro-Gly-X-Gly (X is a variable residue) pentapeptide, undergo a phase transition as a function of temperature. ELPs are soluble in water below their transition temperature, and are insoluble in water and aggregate when the temperature is raised above the transition temperature. We have synthesized a thioredoxin-ELP fusion protein (Trx-ELP), and shown by ellipsometry, surface plasmon resonance spectroscopy, and atomic force microscopy that below its transition temperature, soluble Trx-ELP does not interact with a hydrophobic surface. However, above the transition temperature, insoluble Trx-ELP forms an adsorbed monolayer on a hydrophobic surface, in which Trx is oriented towards the bulk. Adsorbed Trx-ELP binds an anti-thioredoxin monoclonal antibody with high affinity from solution. The Trx-ELP/antibody complex can be resolubilized from the surface by reducing the solution temperature below the transition temperature. The adsorption transition is driven exclusively by the hydrophobic surface, because no adsorption is observed on a hydrophilic surface, and previously aggregated Trx-ELP in solution does not adsorb onto a hydrophobic surface. The adsorbed Trx-ELP complex shows micelle-like organization, with a mean diameter of approximately 100 nm. Current studies on reversible self-organization of ELP fusion proteins onto micro and nano-patterned surfaces, based on an easily controllable solution parameter, suggest their application in biosensor development and modulation of cell-substrate interactions.

9:20am **BI-FrM4 The Fibronectin Type III Domain: A Scaffold for a Molecular Recognition Switch**, ***A. Krammer***, University of Washington; *H. Lu, B. Isralewitz, K. Schulten,* University of Illinois, Urbana-Champaign; *V. Vogel,* University of Washington

The forced unfolding of fibronectin's tenth type III module (FnIII10) was simulated by steered molecular dynamics (SMD) indicating that mechanical tension applied to the module's termini renders its RGD loop inaccessible to cell surface integrins. FnIII10 possesses a beta-sandwich motif consisting of seven beta-strands (A-G) that are arranged in two antiparallel sheets with the RGD peptide sequence located at the apex of the FG loop. Computer simulations now reveal that the b-strand G separates from the module at an early stage of unfolding while the remaining fold experiences only small structural perturbations. Consequently, the RGD peptide is pulled closer to the module's surface as the FG loop unravels. A molecular scale picture of the forced unfolding pathway will be discussed as well as its implications for the understanding of cell-surface interactions.

9:40am **BI-FrM5 Utilizing Direct Communication between Enzymes and Conducting Polymers in Glucose Sensors**, ***A. Kros***, University of Nijmegen, The Netherlands; *S.W.F.M. van Hövell,* TNO Nutrition and Food Research Institute, The Netherlands; *D.M. Vriezema, R.J.M. Nolte,* University of Nijmegen, The Netherlands

Biosensors are currently of great interest because of the potential to measure a variety of substrates like glucose and lactate.@footnote 1@ Glucose is by far the most studied analyte in this field of research, primarely due to its importance in human metabolic processes. Here we report the development of a glucose sensor, which will be utilized to measure in vivo glucose levels in the near future. The working mechanism of the sensor is based upon direct electron communication between the enzyme glucose oxidase and a conducting polymer.@footnote 2@ In this new glucose sensor, ethylenedioxythiophene is polymerized chemically inside the pores of a cyclopore track-etch membrane using iron(III)chloride as a catalyst. In this way, a thin layer of conducting polymer (polyethylenedioxythiophene, pedot) is deposited in the interior of the pores. The latter layer is subsequently covered with the redox enzyme glucose oxidase by means of physical adsorption and electrostatic interactions between the positively charged pedot and the negatively charged enzyme. The resulting sensor is able to detect glucose in the clinical relevant concentration range via amperometric methods. The influence of electrostatic interactions and the use of electronic mediators on the sensor performance will be discussed. @FootnoteText@ @footnote 1@ A.E.G. Cass, A practical approach, Oxford University Press, New York, 1994. @footnote 2@ C.G.J. Koopal, B. de Ruiter, R.J.M. Nolte, J. Chem. Soc. Chem. Commun., 1991, 1691.

10:00am **BI-FrM6 Cap-shaped Gold Nano Particles for Optical Biosensing**, ***M. Himmelhaus***, *H. Takei,* Hitachi, Ltd., Japan

Gold nano particles can be utilized for optical detection of biomolecules.@footnote 1, 2@ The approach presented here is well suited for the development of miniaturized, inexpensive biosensors for two reasons. First, the preparation of the sensing surface is easy to control and highly reproducible. Second, the unique optical properties of cap-shaped gold particles, such as a pronounced reflectivity minimum in the visible region of OD. 2.4 with a bandwidth of 100 nm, lead to a highly sensitive though simple optical read-out quantity. For preparation, a gold layer of 20 nm is first evaporated on a polystyrene (PS) substrate. Then, the gold layer is exposed to an aqueous PS nano sphere suspension containing a small amount of carbodiimide. Addition of the last chemical to the commercially available monodisperse PS sphere suspension leads to formation of a dense monolayer of randomly positioned PS nano spheres on the gold thin film. After one hour of incubation, superfluous PS spheres are simply washed off with deionized water. Finally, a gold layer of 20 nm thickness is evaporated onto the PS sphere monolayer leading to formation of cap-shaped gold particles. The resulting surface exhibits a pronounced extinction peak upon reflection of visible light. The shift of this reflectivity minimum due to changes in the refractive index of the immediate environment can be monitored with simplest optical methods and therefore is well adapted to

miniaturization. We will show that a straightforward fiber-optical setup is sensitive enough to detect in-situ monolayer formation of alkanethiolates. Further, the capability of sensing biomolecular adsorption will be demonstrated utilizing the biotin/avidin functional pair as a model system. @FootnoteText@ @footnote 1@R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger and C. A. Mirkin, Science 277 (1997) 1078-81 @footnote 2@F. Meriaudeau, T. R. Downey, A. Passian, A. Wig and T. L. Ferrell, Appl. Opt. 37 (1998) 8030-7.

10:20am **BI-FrM7 Properties of Thiol Monolayers in Contact with Liquids: An In Situ Investigation by IR-vis Sum Frequency Spectroscopy**, ***M. Zolk***, *F. Eisert, M. Buck, M. Grunze,* University of Heidelberg, Germany

In the vast majority of cases the analysis of the structure and properties of self-assembled monolayers (SAM) takes place ex situ, e.g. in air or even under vacuum. In contrast, the application in fields relevant to biotechnology requires a detailed understanding of their properties in contact with liquids, in particular aqueous solutions. This raises the question of the relevance of ex situ investigations to conditions relevant for applications. We report experiments on SAMs of thiols on gold and metal substrates. The behavior of pure and end group modified thiols in contact with different liquids is studied. In particular, we focus on SAMs which consist of a methoxy terminated ethylene glycol (EG) unit attached to an alkane thiol. Whereas SAMs of pure alkane thiols are affected relatively little by solvents, EG-SAMs which are important for the preparation of protein resistant surfaces, exhibit a drastic dependence on the environment. All solvents examined penetrate the layer and induce significant conformational changes. Compared to air the signal from the methoxy end group is strongly reduced in contact with solvents. This indicates a transition from a well-ordered to an amorphous state. Analogously, the methylene vibrations gain intensity and thus indicate an increase of gauche conformations. Depending on the solvent the degree of penetration is different. A polar solvent such as water mainly interacts with the EG part of the layer whereas non-polar solvents interact as well with the hydrocarbon chain and thus penetrate deeper into the SAM. The experiments clearly demonstrate the need to investigate SAMs under conditions relevant for applications.

10:40am **BI-FrM8 Biofilm - Titanium Chemistry of Adhesion Using X-ray Photoelectron Spectroscopy**, ***R.A. Brizzolara***, NSWC - Carderock Division

Virtually all surfaces immersed in water for any period of time are colonized by microorganisms. These organisms adhere to the surface by producing extracellular polymers, predominantly polysaccharides. Biofilm formation and resulting biofouling cause serious problems for heat transfer equipment due to inhibited water flow and degradation of the heat transfer coefficient. Conventional coatings cannot be applied to heat transfer materials due to degradation of the heat transfer coefficient. Titanium, often the material of choice for heat transfer applications because of its corrosion resistance, is very prone to biofouling. Materials and/or surface modification strategies to decrease the strength of adhesion or the rate of biofilm formation would be of great value. As a first step in developing such a strategy, the interfacial chemistry between biofilm components and titanium is being investigated. This paper reports on the use of x-ray photoelectron spectroscopy to examine the interfacial chemistry between alginic acid and n-acetyl glucosamine and titanium. XPS is used to quantify the adsorbate bound to the surface under various conditions (including pH and salt content of the water), and to evaluate the adsorbate-surface bonding mechanism. Information regarding the biofilm-surface chemical interaction will be useful in developing better fouling resistant surfaces. The NSWC Carderock Division In-House Laboratory Independent Research Program supported this work.

11:00am **BI-FrM9 Planar Polymerized Phospholipid Bilayers as Biocompatible Substrates**, ***J.C. Conboy***, *S. Liu, D.F. O'Brien, S.S. Saavedra,* University of Arizona

There is considerable interest in finding a surface that is resistant toward non-specific protein adsorption and is chemically and mechanically stable. Hydrophilic surfaces, such as those of a zwitterionic phospholipid bilayer, are inherently biocompatible with intrinsically low nonspecific protein interactions. However, planar supported lipid bilayers are only weakly associated, making their stability less then desirable from an applications standpoint. Toward the goal of producing a stable and intrinsically biocompatible substrate, we endeavored to produce planar polymerized analogs of phospholipid bilayers. A photosensitization method was used to polymerize the lipid bilayers in aqueous media. The rate of polymerization and subsequent structural changes in the lipid film were examined by in-situ Raman spectroscopy. The stability of the lipid films was determined before and after in-situ polymerization by a number of methods. Their application as substrates for optical biosensors will also be discussed.

11:20am **BI-FrM10 Nanoscale Patterning of Gold for Attachment of Supported Lipid Bilayers**, ***A.T.A. Jenkins***, Max-Planck Institut für Polymerforschung, Germany

Attaching lipid bilayers to solid substrates in such a way that they exhibit properties analagous to cell membranes found in Nature is becoming of increasing interest. Such systems have the potential to be used as biosensors and for fundamental studies of cell membranes. In this paper we present a novel method of attaching such lipid bilayers to gold substrates using microcontact printing to produce a patterned surface of sub-micron size patterns onto which a lipid layer is added. Microcontact printing has been used to form patterns of lipophilic Self-assembled monolayers (SAMs) on gold with dimensions of 500 nm or less. These patterns consist of a regular array of hydrophilic and hydrophobic patches. Onto these patterned SAMs, lipid bilayers have been formed over the hydrophilic patches by lipid vesicle rupture and self-assembly. Investigation of lipid bilayers on these small nanometer scale patterns compared with larger micrometer scale patterns of lipopilic SAMs, by both Impedance Spectroscopy and Surface Plasmon Spectroscopy have suggested that vesicle adsorption followed by rupture at hydrophilic-hydrophobic SAM interfaces may be a crucial part of the mechanism of bilayer formation on such patterned SAMs. Finally, ion-selective peptides and proteins including Valinomycin and Gramicidin have been inserted into the bilayer patches, and the expected ion-selectivity observed experimentally.

Surface Science Division
Room 606 - Session SS1+AS+BI-FrM

Organic Films/Self-Assembled Monolayers

Moderator: G.E. Poirier, National Institute of Standards and Technology

8:20am **SS1+AS+BI-FrM1 Simple Viscosity Model Analysis of Hydronium Ion Motion in Nanometer Organic Films**, ***K. Wu***, *M.J. Iedema, J.P. Cowin,* Pacific Northwest National Laboratory

Nanometer organic films such as methylcyclohexane and 3-methylpentane were vapor-deposited at 30 K on Pt(111) with a molecular beam. Pure hydronium ions were soft-landed on top of the films at a kinetic energy less than 1.2 eV. The voltage change and desorption of the organic films were simultaneously monitored by a Kelvin probe and a mass spectrometer. Ion dosing on the organic films was a capacitive charging process, therefore the film voltage change actually reflected the ion motion in the organic films, assuming the dielectric constants of the organic films do not change much with temperature. When the films were warmed, ions gradually moved into the films. The experimental results were analyzed by a simple viscosity model. To a large extent, the temperature (or time) evolution of the film voltage could be well predicted by the model. The film voltage fall-off temperature width from the theory was, however, about half of that from the experiment. Further experimental evidence showed that the ion self-generated electric field strength had a strong effect on the ion motion. For example, when the electric field strength was higher than 0.05 V/Angstrom, the theoretical prediction seriously deviated from the experimental result, indicating that a high electric field might trigger non-linear ion motion and made the Stokes-Einstein equation not accurate. When films were prepared at higher temperatures, methylcyclohexane could crystallize on Pt(111), making ions more difficult to transport in the crystalline films. But, 3-methylpentane never crystallized before ion motion in it completed, indicating that it's a good glass material. With this general approach, we could attack many important issues such as ion motion across liquid-liquid interfaces, hydration of ions and so on.

8:40am **SS1+AS+BI-FrM2 Direct Observation of Topological Defect Evolution and Domain Motion in Ultrathin Films of PS-b-PMMA Diblock Copolymers Using Atomic Force Microscopy**, ***J. Hahm***[1], *W.A. Lopes, H.M. Jaeger, S.J. Sibener,* The University of Chicago

We report the tracking of individual topological defects in the microdomain patterns of cylinder-forming polystyrene-block-polymethylmethacrylate (PS-b-PMMA) films. These films undergo vertical and lateral phase separation when they are thermally annealed. The vertical phase separation results in thickness quantization where each layer exhibits its own topology and dynamics. The lateral phase separation provides height contrast between the two components of the diblock in single-cylinder-

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

layer thick films. In the atomic force microscopy (AFM) topographic images, the PMMA is higher by approximately 1nm as compared to the PS blocks. 50nm thick films, containing a single layer of cylinders aligned parallel to the film plane, were repeatedly and non-destructively probed with AFM in an attempt to elucidate the evolution of the diblock domain topology between annealing treatments. We show explicitly that the evolution of topological defects takes place through relinking, joining, clustering and annihilation of defects. Such processes form the basis for predicting structural changes in polymer thin films. We also have used time-lapse AFM imaging to observe directly the kinetics of domain mobility responsible for topological evolution. Domains of different thicknesses were monitored as a function of annealing temperature and time. The higher mobility and lower activation energy associated with thicker domain mobility are accounted for by the essentially negligible substrate interactions where polymer-polymer rather than polymer-substrate interactions govern the dynamics. Our hope is that the combined understanding of topological changes, such as those reported in this talk,when combined with mobility kinetics, will give us a predictive understanding of the thermally activated structural changes that occur within thin polymer films. @FootnoteText@ Supported by the NSF-MRSEC at the University of Chicago and AFOSR.

9:00am **SS1+AS+BI-FrM3 Properties of Self-Assembled Monolayers of Biphenyl-Based Thiols**, ***T. Felgenhauer***, *H.-T. Rong, M. Buck, M. Grunze,* University of Heidelberg, Germany

Despite their versatility to modify surface properties, self-assembled monolayers (SAM) based on alkane thiols have limitations concerning conformational stability or structural perfection. In search for more rigid molecules, thiols based on aromatic moieties offer an alternative. However, in contrast to SAMs consisting of alkane thiols experiments on aromatic thiols are relatively scarce. Our experiments focus on thiol SAMs consisting of 4,4'-substituted biphenyls (BP). To allow systematic investigations the number of methylene units between the biphenyl moiety and the thiol group was varied between zero and six. The electrochemical behavior of BP-SAMs turns out to be very different from alkane thiols. In general, the charge permeability of BP-SAMs is higher by orders of magnitude even though the electrochemical stability of BP-SAMs is dependent on the methylene spacer. Exposure to an etching solution reveals a stability significantly higher compared to alkane thiols and suggests an improved structural perfection of BP-SAMs. Spectroscopic characterization of the BP-SAMs yields an orientation of the biphenyl units alternating with the methylene chain length. Comparison of BP-SAMs on Au with those on Ag shows a reversal of the odd-even effect and demonstrates that the sulphur-substrate bond is crucial for the molecular orientation of the biphenyl-SAMs.

9:20am **SS1+AS+BI-FrM4 Structure of Self-assembled Monolayers of Alkanethiols and Disulfides on Au(111)**, ***H. Nozoye***, National Institute of Materials and Chemical Research, Japan*; C. Kodama, T. Hayashi,* University of Tsukuba, Japan

Self-assembled monolayer (SAM) films of alkanethiols and alkyldisulfides have been attracting an increasing interest. However, we do not have a concrete picture of SAM, e.g. is the SH bond broken on the surface ? , is the SS bond formed on the surface ?, or where is the adsorption site of S ?. We studied these problems by means of STM, HREELS, high-sensitivity LEED, and TPD. Alkanethiols (C1-C10) and alkyldisulfides (C2-C12) were adsorbed on a Au(111) single crystal surface at room temperature or at around 120 K. Dersorption of H2, parent alkanethiol, alkylthiolate radical, and alkyldisulfide were observed in TPD and a vibration peak assigned to a SS bond was missing in HREEL spectra. We clearly concluded that the SH bond of alkylthiols breaks at low temperature forming alkylthiolates on the surface. Furthermore, the SS bond of alkyldisulfides was shown to be broken on the surface, forming alkylthiolate. The Au-S bond of alkylthiolates on the surface gave a relatively strong peak in HREEL spectra. We will discuss the formation process and the relation between the local structure and the long-range order of SAM.

9:40am **SS1+AS+BI-FrM5 Toward Vapor Deposition of Polycyanurates: The Surface Chemistry of Phenyl Cyanate and Phenol on Al(111)**, *B. Bartlett, J.M. Valdisera,* ***J.N. Russell, Jr.,*** Naval Research Laboratory

Polycyanurates, formed by polymerization of monomers containing two cyanate groups, show promise as vapor depositable, low dielectric parameter materials for microelectronics applications. Consequently, we are examining the chemistry of a model system, phenyl cyanate on Al(111) surface, with temperature programmed desorption (TPD), Auger electron spectroscopy (AES), and X-ray photoelectron spectroscopy (XPS). (Aluminum was chosen as a substrate because it is used for interconnects in microelectronics.) For submonolayer coverages, XPS shows that the phenyl cyanate undergoes O-CN bond cleavage between 150 and 200 K, leaving phenoxide and cyanide groups on the surface. Benzene and hydrogen decomposition desorption products were observed at 620 K, and between 500 and 800 K, respectively. This is analogous to the reaction of phenol on Al(111). Deuterium labeling was used to determine the sequence of C-H bond scission on the phenoxy species. XPS and AES reveal the formation of aluminum oxide, nitride and carbide on the surface above ~ 400 K. After multilayer exposures, the formation of the phenyl cyanate trimer, triphenoxytriazine, was observed with XPS between 160 and 200 K. The trimerized multilayer was stable up to ~ 500 K before it decomposed. This data suggests that at high coverages, a dicyanate functionalized molecule may chemically attach to aluminum by cleavage of an O-CN bond, while the other cyanate group is available for trimerization, thus anchoring a polycyanurate film to the surface. The surface chemistry of a dicyanate functionalized molecule, 1,1-bis(diphenylcyanato)ethane, is currently under investigation to confirm this hypothesis.

10:00am **SS1+AS+BI-FrM6 Formation of Organic Layers by Cycloaddition Reactions at Germanium Surfaces**, *S.W. Lee,* University of Missouri, Columbia*; J.S. Hovis, R.J. Hamers,* University of Wisconsin, Madison*;* ***C.M. Greenlief***, University of Missouri, Columbia

The formation of ordered organic layers on Ge(001) substrates is explored. Ge substrates are prepared by a novel method and exposed to a variety of cyclic hydrocarbons, including cyclopentene and cyclohexene. The subsequent surface interactions are followed by a variety of surface sensitive techniques. Bonding configurations are determined by photoelectron spectroscopy and scanning tunneling microscopy. The strength of surface interactions are also monitored by temperature programmed desorption. Cyclopentene and cyclohexene react with Ge dimer bonds via a [2+2] cycloaddition reaction. This reaction generates rows of the surface complex oriented along the dimer bond direction of the Ge(001) surface, which is easily observed by scanning tunneling microscopy. Experiments using other cyclic hydrocarbons and attempts at further surface modification will be discussed.

10:20am **SS1+AS+BI-FrM7 Multilayer Phases in Self-Assembled Monolayers Based on Silane Coupling Agents**, ***B.C. Bunker***, *R.W. Carpick, M. Hankins, M.L. Thomas, R. Assink, M. DeBoer,* Sandia National Laboratories

Thin films prepared using silane coupling agents are used extensively to chemically modify surfaces. In micromachines, such films are used to control stiction, friction, and adhesion of moving parts. The films are commonly depicted as self-assembled monolayers, in which each silane molecule forms extensive Si-O-Si linkages to the surface and to other molecules. However, many workers report that it is difficult to produce self-assembled monolayers on a reproducible basis, especially for films having fluorinated hydrocarbon chains. In this paper, atomic force microscopy studies are used to show that irreproducible film formation is associated with the fact that silane coupling agents can self-assemble into a range of structures described in common surfactant phase diagrams. Evidence is presented suggesting that hydrocarbon and fluorocarbon silanes form lamellar and inverse micelle structures on silica and silicon nitride surfaces. In some instances, multilayer structures are produced during fabrication. Films which start out as monolayers can also reorganize into multilayer phases after deposition. Factors influencing the phases observed include surface pretreatment, the solvent, silane and water concentrations in the deposition solution, and environmental parameters such as temperature and relative humidity. Mechanisms for the evolution of the observed range of self-assembled structures vs. reaction conditions are described.

10:40am **SS1+AS+BI-FrM8 Self-assembled Monolayers on Silicon Surfaces: The Opposite to Siloxane Chemistry**, ***J.A. Mulder***, *R.P. Hsung, X.-Y. Zhu,* University of Minnesota

Self-assembled monolayers (SAMs) on silicon surfaces are of interest for a number of reasons: they may be used as monolayer resists in high resolution lithography, as dielectric layers, as active components in hybrid sensor devices, as passivation and lubrication layers in MEMS, and as a new platform for biochips. SAMs on silicon are traditionally formed via siloxane chemistry on oxidized surfaces from organosilicon derivatives, such as alkyltrichlorosilane. The problems with siloxane SAMs are well known: they are difficult to prepare and suffer from poor reproducibility; the contradiction between cross-linking and close-packing is inherent; the presence of the amorphous and insulating oxide layer is not desirable in

some applications. There is much incen tive to develop simple processes for the formation of stable molecular layers directly on the silicon surface. We present a novel approach which, in essence, is the opposite to the siloxane SAM process. The assembly processes are based on the reaction bet ween R-OH or R-NH2 with chlorinated silicon surfaces, leading to molecular assembly via Si-O or Si-N linkages. These reactions are not only efficient but also sufficiently versatile for the assembly of a wide variety of functional organic molecules. A par ticular advantage of this new assembly chemistry is that it is compatible with both vacuum and solution phases and can be carried out under very benign experimental conditions. We characterize these SAMs using a variety of techniques, such as multiple-int ernal reflection FTIR, X-ray photoelectron spectroscopy, contact angle measurements, and scanning probe microscopy. We address structural and stability of these SAMs and their dependence on molecular structure, such as alkyls and aromatics. We also discuss immediate and future applications.

11:00am **SS1+AS+BI-FrM9 The Photochemistry of Model Organosulfur Compounds Adsorbed on GaAs (110): Energy-Resolved Photofragment Angular Distributions***, **N. Camillone III**, K. Adib, R.M. Osgood, Jr.,* Columbia University

Self-assembled monolayers of organothiols and related compounds hold promise for use in the semiconductor industry as ultrathin electron beam resists and precursors for the growth of II-VI materials, as well as for the growth of passivating gallium sulfide films on GaAs. An understanding of the structure and photon and electron chemistry of organothiols and related compounds is relevant to the development of these technologies. We report on our studies of the photoinduced chemistry of three model organosulfur compounds, CH@sub 3@SH, (CH@sub 3@S)@sub 2@ and CH@sub 3@SCH@sub 3@, on the (110) surface of GaAs. We find that the cross sections for the photoinduced reactions of these molecules in the adsorbed monolayers follows the trend CH@sub 3@SH > CH@sub 3@SCH@sub 3@ > (CH@sub 3@S)@sub 2@. Comparison of these measurements to photoinduced chemistry in the multilayer regime gives insight into the degree to which close proximity to the semiconductor surface perturbs the photochemistry. In addition, the structure and molecular orientation of these molecules is discussed in light of the results of energy-resolved photofragment angular distributions and low energy electron diffraction measurements. The post-irradiation chemistry of the trapped photofragments will also be discussed.

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