Biomaterials
Room 102 - Session BI-MoM

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

9:40am **BI-MoM1 Chemical Recognition on Lipid Membrane Surfaces*, D.Y. Sasaki,*** Sandia National Laboratories **INVITED**

Cell communication and sensing are processes mediated by chemical recognition events that occur on the lipid membrane surface. Chemical signals in bulk solution are recognized by membrane receptors, which subsequently organize into specific structures that activate signal cascades. Through a biomimetic approach, we have examined chemically induced molecular reorganization events in lipid membranes in an effort to learn how to control this process for sensor applications and nanoscale architecture. By using a synthetic approach we have simplified interactions between molecular species to evaluate their effects on molecular reorganization. Simple two-component lipid bilayer assemblies were prepared with receptors for heavy metal ions, proteins, and polypeptides. The aggregational state of these fluorophore-labeled receptor molecules, as they responded to chemical recognition events, were monitored globally by spectroscopic means (e.g., excimer formation of pyrene labels) and locally via in situ atomic force microscopy (AFM). We found that the dispersion and aggregation of receptors in a bilayer can be directed through multiple levels of interactions, such as electrostatic charge from metal ion chelation, multiple-point binding interaction with polyfunctional guests, and phase separation. In situ AFM studies observed that nanoscale structures composed of aggregated receptors but could be made to disappear or reappear upon the addition or removal of specific chemical ligands. At a slightly larger scale, the functionalized bilayers displayed a unique ability to self-assemble into hierarchical structures of stacked bilayers through a process mediated by chemical recognition. These lipid bilayers with their unique optical response, biocompatibility, and self-organizational properties demonstrate a versatile array of possibilities for sensing and nanoarchitecture.

10:20am **BI-MoM3 Surface Docking Sites for Macromolecules: Interface Architecture based on PLL-g-PEG/PEGbiotin-(Strept)avidin*, N.-P. Huang,*** *J. Vörös, S.M. De Paul, M. Textor, N.D. Spencer,* ETH Zürich, Switzerland

Surface docking sites (nanoscale islands) are desirable for the specific adhesion of macromolecules, such as proteins and oligonucleotides, onto surfaces. The surrounding areas of such docking sites should be non-adhesive so that the adsorbed macromolecules are prevented from denaturing after adsorption. We have mixed poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) and a variant of PLL-g-PEG in which some of the PEG chains are biotinylated (PLL-g-PEG/PEGbiotin) to form a novel polymeric interface, in order to tailor chip surfaces in terms of non-specific and specific analyte-surface interactions. By means of optical waveguide lightmode spectroscopy (OWLS), streptavidin and avidin are shown to bind specifically to the biotin-functionalized PEG, while the resistance of the remaining PEG chains to protein adsorption yields a high specific-binding-to-non-specific-binding ratio. Subsequent binding of biotinylated goat-anti-rabbit immunoglobulin (@alpha@RIgG-biotin) to (strept)avidin as a capture molecule allows the system to be used as an immunoassay for the target molecule, rabbit immunoglobulin (RIgG). Changing the ratios of PLL-g-PEG and PLL-g-PEG/PEGbiotin in the mixture changes the distribution of docking sites (biotin sites) on the interface and, thus, allows optimization of the sensing response. The effects of protein charge and the ionic strength of the buffer are also explored. We expect that such a platform could serve as a powerful tool for the investigation of molecular recognition effects.

10:40am **BI-MoM4 Self-Assembly of Modified Porphyrin Monolayers*, A.L. Bramblett,*** *M.S. Boeckl, K.D. Hauch, T. Sasaki,* University of Washington*; J.W. Rogers, Jr.,* Pacific Northwest National Laboratory*; B.D. Ratner,* University of Washington

Assembled monolayers can be used to deliver recognition signals to biological systems. Planar, multi-ring porphyrin structures can provide precision lateral control of chemistry at interfaces. Porphyrin molecules, which have peripheral constituents with the ability to hydrogen bond or coordinate to a metal, are predicted to form ordered molecular monolayers on gold surfaces in a side-by-side orientation. TPyP-Ge-Disulfide (a Ge coordinated pyridyl substituted porphyrin with two disulfide ligands) has been synthesized for use in self-assembling porphyrin monolayers, which form when one of two disulfide ligands binds to the gold surface. Self-assembled monolayers of TPyP-Ge-disulfide have been prepared on gold for characterization. X-ray photoelectron spectroscopy (XPS) of TPyP-Ge-disulfide shows that the atomic composition of the surface is consistent with a porphyrin monolayer, and approximately 50% of the sulfur atoms are bound to gold. Further, ultraviolet-visible spectroscopy (UV/Vis) shows a red shift in the peak of the Soret band, which is indicative of side-by-side porphyrin orientation when self-assembled. @footnote 1@ In addition, monolayers formed utilizing hydrogen bonding between the TPyP-Ge-disulfide and TCP (benzoic acid derivatized porphyrin), or utilizing Zn@super 2+@ coordination with the pyridyl nitrogen in TPyP-Ge-disulfide will be characterized using XPS, UV/Vis, and scanning tunneling microscopy, to examine the porphyrin arrangement on the gold surface. By chemically modifying the remaining free disulfide bond, these monolayers could be used to present biological ligands of interest in a spatially controlled manner. @FootnoteText@ @footnote 1@ Boeckl, M. S.; Bramblett, A. L.; Hauch, K. D., Sasaki, T.;Ratner, B. D.; Rogers, Jr, J. W. Langmuir 2000, 16, 5644-5653

11:00am **BI-MoM5 Efficient New Method of Nucleic Acid Immobilization*, Y. Wu,*** *P.L. Dolan, L.K. Ista, M.A. Nelson,* University of New Mexico*; R.L. Metzenberg,* Stanford University*; G.P. Lopez,* University of New Mexico

The field of DNA microarray technology has necessitated the cooperative efforts of interdisciplinary scientific teams to achieve its primary goal of rapidly measuring preferential gene expression in an organism. To that end, a collaborative effort to produce a chemically reactive surface on glass slide substrates to which DNA will covalently bind for use in and advancement of cDNA microarray technology was undertaken. We have developed a chemical process for covalently linking unmodified DNA to an ordinary microscope slide in a manner that preserves the ability of the immobilized nucleic acid to hybridize to complementary sequences. The method of binding DNA to solid surfaces considerably increased the consistency and uniformity of attachment, and reduced DNA loss during the experimental process when compared to other commonly used commercially available methods. Moreover, better hybridization results have been generated compared to commercially available immobilization techniques. In general, this method allows binding of single- and double-stranded nucleic acids onto a solid substrate that can lead to considerable improvements in hybridization of complementary sequences, stability of affixed DNA, and re-usability of microarrays. Following our immobilization process, arrayed slides were reusable for at least 5 times. In addition, the hybridization data has been analyzed quantitatively and successfully correlated with solution concentrations. Although this method is originally designed for forming DNA microarrays, it is likely also suitable for the immobilization of proteins, ribozymes and aptamers onto certain solid substrates..

11:20am **BI-MoM6 Probe Density Effects for Target Hybridization in DNA Monolayer Films Studied By SPR*, A.W. Peterson,*** *R.J. Heaton, L.K. Wolf,* Boston University*; R.M. Georgiadis,* Boston University, usa

Understanding probe to target interactions for surface immobilized duplexes is important in the emerging applications of DNA biosensors. We use in-situ surface plasmon resonance spectroscopy to monitor the kinetics of probe immobilization and target/probe hybridization for thiol-modified probes immobilized on gold. We find that both the efficiency and kinetics of probe/target hybridization depend strongly on probe density. Immobilization conditions can be used to control probe density and we investigate the effects of solution ionic strength, electrostatic potential at the interface and whether duplex or single stranded probes are immobilized. Independent of which immobilization strategy is used, we find that DNA films of equal probe density exhibit reproducible efficiencies and kinetics for probe/target hybridization. In addition, the hybridization isotherms show a distinct dependence on probe density. Insight into the mechanism of these probe/target interactions are investigated and discussed in the context of the observed kinetics.

11:40am **BI-MoM7 Two-dimensional Arrangements of Streptavidin on Biotin-doped Supported Lipid Bilayars Optimized for DNA-DNA Hybridization*, C. Larsson, E. Fridell, B. Kasemo, F. Höök,*** Chalmers University of Technology, Sweden

In this work the quartz crystal microbalance with dissipation monitoring (QCM-D), surface plasmon resonance (SPR) and ATR-FTIR techniques were used to follow the 2-D arrangement of streptavidin on a biotinylated phospholipid bilayer supported on a SiO2 surface, for optimized capture of single stranded biotin-DNA (15- and 30-mer) and subsequently hybridization-kinetics measurement of their reaction with mixed-sequences of DNA (15- and 30-mer) with various degrees of mismatch. The

QCM-D and ATR-FTIR data suggest that streptavidin rearranges into a more rigid and oriented structure at approximately 50 % coverage, interpreted as onset of crystallization. Interestingly, immobilization of biotin-DNA followed by subsequent hybridization prior to this stage results in up to a two-fold more rapid association kinetics compared with immobilization and hybridization at full coverage of streptavidin. Our results demonstrate how real-time control of the immobilization step can be efficiently used to minimize the influence from lateral interactions for surface-based biorecognition detection, and thus optimize hybridization kinetics measurements. The QCM-D data were also used to quantify variations in the viscoelastic properties of the formed layers of DNA and DNA duplexes, which were further correlated to the obtained results onhybridization kinetics.

Biomaterials
Room 102 - Session BI+SS-MoA
Role of Water in Biological Systems

2:00pm **BI+SS-MoA1 Simulation Studies of the Structure and Dynamics of Biological Hydration Water**, ***D.J. Tobias,*** University of California, Irvine; *M. Tarek,* National Institute of Standards and Technology **INVITED**

Water, life's solvent, plays two vital roles in the function of biological macromolecules and their assemblies. One is to stabilize the specific structures that these molecules maintain in their functioning states. Another is that fast movement of water molecules promotes the flexibility of biological molecules required for their function. I will present results from molecular dynamics simulations that illustrate two peculiar aspects of water structure and dynamics near biological molecules. The first is the structure of water on the surface of lipid membranes. I will show that the first layer of water coating the membrane surface is strongly influenced by the lipid molecules, and that the anisotropic solvation of the lipid polar groups profoundly affects the polarity of the membrane/water interface. The second is the motion of water molecules on the surface of proteins. Below a threshold level of hydration and thermal energy, proteins exist in an inactive, glassy state. I will show that the transition from the glassy state to the functioning state requires relaxation of the protein-water hydrogen bond network. Finally, I will discuss some of the anomalous dynamical properties of protein hydration water, drawing analogies with supercooled and confined water.

2:40pm **BI+SS-MoA3 Local Solvation Shell Measurement in Water using a Carbon Nanotube Probe**, ***S.P. Jarvis,*** Nanotechnology Research Institute, AIST, Japan; *T. Ishida,* Institute for Mechanical System Engineering, AIST, Japan; *C.C. Liew,* Research Institute for Computational Sciences, AIST, Japan; *H. Tokumoto,* Nanotechnology Research Institute, AIST, Japan., Japan; *Y. Nakayama,* Osaka Prefecture University, Japan **INVITED**

Using a multiwalled carbon nanotube as an atomic force microscope (AFM) probe tip we have directly measured localised structuring in aqueous environments at small tip-sample separations and have combined this with nanometer resolution images of the surface. By diversifying beyond the simple surfaces of graphite and mica, to self-assembled monolayers with varying end groups, we have been able to investigate the role of local surface chemistry and morphology on the measured water structure. Directly measuring solvation shells with a mechanical probe of lateral dimensions comparable to that of a single molecule provides an invaluable insight into the processes controlling if and how a molecule approaches another molecule or a membrane. In the immediate vicinity of the molecule, continuum models break down and the aqueous environment will often form a discrete layered structure depending on the nature of the molecule. The absence or presence of such structure may be fundamental in influencing the promotion or inhibition of protein adsorption, biological function and membrane recognition. In order to perform such measurements it has been necessary to combine a number of innovative techniques with a standard AFM. For high-resolution imaging we use a highly sensitive frequency modulation detection scheme. To do this effectively in liquid involves the implementation of magnetically activated dynamic mode (MAD-mode!) where a small magnetic particle is attached to the end of the cantilever and an external magnetic field applied via a current carrying coil. To increase the sensitivity of the measurement to the interaction local to the tip apex we have used a high aspect ratio multiwalled carbon nanotube probe. This reduces the hydrodynamic squeeze damping between the surface and the bulk of the tip. The nanotube is attached in a specially designed field emission scanning electron microscope, which permits us some control over both the length and direction of the probe.

3:20pm **BI+SS-MoA5 The Role of Interphase Water in Protein Resistance**, ***J.G. Kushmerick,*** *J.E. Houston, B.C. Bunker,* Sandia National Laboratories

While the inertness of oligo(ethylene glycol) (OEG) terminated self-assembled monolayers (SAMs) towards protein adsorption is well documented, the physical cause for the protein resistance has remained the subject of debate. Steric repulsion, which accounts for the inertness of endgrafted poly(ethylene glycol), is not applicable to thin densely packed OEG-SAMs. A strongly bound water layer templated by the OEG-SAM has been proposed to account for the protein resistance. Interfacial force microscope measurements of the interaction between functionalized probe tips and OEG-SAMs in water reveal a long-range (> 4 nm) repulsion. The repulsion is consistent with the existence of a thick interphase water layer with an elastic modulus similar to that of ice. Such an interphase layer, which is consistent with theoretical calculations and neutron reflectivity data, could account for the protein resistance of OEG-SAMs. Experiments aimed to further understand the mechanical properties of the water interphase, including varying the metal substrate, variable temperature and quartz crystal microbalance measurements, will also be discussed. Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed-Martin Company, for the US Department of Energy.

3:40pm **BI+SS-MoA6 Computer Simulation of the Behavior of Water near Model Surfaces and Self-Assembled Monolayers**, ***A.J. Pertsin,*** *T. Hayashi, M. Grunze,* Heidelberg Universität, Germany **INVITED**

The Grand canonical ensemble Monte Carlo technique is used to simulate the behavior of the TIP4P model of water confined between two parallel hydrophilic or hydrophobic model surfaces and also the surfaces of oligo(ethylene oxide) (EGn) terminated self-assembled monolayers (SAMs). The interaction of water with hydrophobic surfaces is modelled by a conventional (3-9) potential dependent only on the separation of the water oxygen atom from the surface. The model potential for hydrophilic surfaces involves in addition an orientation dependence of the potential well depth, which allows for the orientation of the water hydrogens and lone pairs with respect to the proton-acceptor and/or proton-donor centers on the model surface. The water-SAM interactions are described using an atomistic force field based on ab initio MP2 level results for small EGn-water complexes.@footnote 1@ The transferability of the force field on the particular EGn chains constituting the SAM is tested by comparing the force field predictions with the relevant ab initio DFT results.@footnote 2@ The effect of the surfaces on the contiguous water is analyzed in terms of hydration pressure, average water density, and various distribution functions characterizing the orientational and positional order in water. The simulated water density distribution near the SAM is in good agreement with recent neutron reflectivity measurements which reveal the existence of a fairly thick (c.a. 50 Å) interphase water layer with a noticeably reduced average water density (85-90 % bulk water density).@footnote 3@ @FootnoteText@ @footnote 1@ D. Bedrov, M. Pekny, G. D. Smith, J. Phys. Chem. B 102, 996 (1998). @footnote 2@ R. L. C. Wang, H. J. Kreuzer, M. Grunze, Phys. Chem. Chem. Phys. 2, 3613 (2000). @footnote 3@ D. Schwendel et al., submitted.

4:20pm **BI+SS-MoA8 The Water Content of Proteins during Adsorption**, ***J. Voros,*** *M. Textor, N.D. Spencer,* ETH-Zurich, Switzerland

Adsorption of proteins at solid-liquid interfaces is a process of central importance for biosensors and biomaterials. The role of water is a key issue in this process. The use of two biosensor techniques and identical experimental conditions have made it possible to follow the evolution of the water content of proteins during the surface adsorption-relaxation process.@footnote 1@ Optical waveguide lightmode spectroscopy (OWLS) involves the incoupling of a laser into a planar waveguide generating an evanescent field. The measurement of the incoupling angles allows for the online monitoring of the dry mass of surface-adsorbed macromolecules. Quartz crystal microbalance with dissipation factor (QCM-D) is a technique for monitoring the mass of adsorbed molecules via changes in the resonant frequency, f, while also getting information about the viscoelasticity of the layer by measuring the dissipation factor, D. The f-shift of the QCM-D is due to the change in total coupled mass, including the water coupled to the layer. The amount of water in an adsorbed adlayer can thus be determined by subtracting the adsorbed mass value obtained by OWLS from the value measured by the QCM-D in experiments carried out under identical conditions. The water content of the protein layer was found to be characteristic for different proteins and to change during the adsorption process. The time evolution of the water content provides information on the conformational changes during the adsorption. The dissipation factor measured by the QCM-D correlates well with the amount of water present in the adsorbed protein layer. Several blood proteins were measured on hydrophilic (TiO@sub 2@) and on hydrophobic surfaces. Dependence on the protein concentration and on the ionic strength of the buffer was also examined. @FootnoteText@ @footnote 1@ F. Hook, J. Voros, M. Rodahl, R. Kurrat, P. Boni, J.J. Ramsden, M. Textor, N.D. Spencer, P. Tengvall, J. Gold, B. Kasemo, Colloids and Surfaces B: submitted, 2000.

4:40pm **BI+SS-MoA9 X-Ray Absorption Spectroscopy of Liquid and Gaseous Water**, ***K.R. Wilson**, R.J. Saykally,* University of California-Berkeley*; J.G. Tobin,* Lawrence Livermore National Laboratory

X-ray absorption fine structure (XAFS) measurements have been performed upon liquid@footnote 1@ and gaseous@footnote 2@ H2O. Using the O1s level as the means of achieving elemental specificity, both near edge (NEXAFS) and extended X-ray absorption fine structure (EXAFS) have been measured. Liquid water samples were achieved in the vacuum system via the utilization of a liquid jet system modelled after that of Faubel et al.@footnote 3@ In the investigation of liquid water, both ions and electrons were used as a means of detection. This permitted the separation of liquid surface effects (ions) from bulk-like behavior (electrons). In the NEXAFS regime, the surface sensitive spectrum resembled that of gaseous water while the bulk-sensitive spectrum exhibited broadening and a blue shift. Similarly, differences were observed in the EXAFS results derived from each detection method, i.e. surface vs. bulk. The measurement of the EXAFS in liquid water encouraged us to go back and perform similar measurements upon gaseous water. A single oscillation was observed from gaseous water consistent with the location of the covalently bonded hydrogen in H2O. The experimental phase and amplitude of the oscillation are in excellent agreement with curved wave multiple scattering calculations for isolated water molecules, performed by Ankudinov and Rehr.@footnote 2@ With this determination of the O-H scattering phase shift , the covalent hydrogen bond distance (0.95 + 0.03 Ã…) in liquid water has been quantified, thus demonstrating that hydrogen EXAFS can become a valuable complement to existing structural methods in chemistry and biology. @FootnoteText@ @footnote 1@K. R. Wilson, et al, J. Chem. Phys. B, May 2001. @footnote 2@K. R. Wilson, et al, Phys. Rev. Lett. 85,4289 (2000). @footnote 3@M. Faubel et al, J.Chem. Phys. 106, 9013 (1997).

5:00pm **BI+SS-MoA10 Proton Dynamics in Ice: A Resonant Photoemission Study**, ***D. Nordlund**,* Uppsala University, Sweden*; M. Cavalleri,* University of Stockholm, Sweden*; H. Ogasawara, L.-Å. Näslund, M. Nagasono,* Uppsala University, Sweden*; L.G.M. Petterson,* University of Stockholm, Sweden*; A. Nilsson,* Uppsala University, Sweden and Stanford University, Sweden

We have studied resonant photoemission around the O-K edge of ice. There is an interference effect between the direct photoemission and core decay processes seen in the valence orbitals. The most striking results is a binding energy shift of 0.5-1 eV of the valence states upon excitation into a core exciton at the bottom of the conduction band in ice. The shift in the orbital energies can be related to motions of the proton to the neighbouring water molecule connected through a donor H-bond during the lifetime of the core hole. This will give us a probe to study proton dynamics in H-bonded system on a femtosecond time scale. We are currently computing the potential of the proton in the core-excited state using DFT.

Biomaterials
Room 134/135 - Session BI-MoP
Biorecognition Poster Session

BI-MoP1 Silica-Elastin Like Polypeptide Smart Membranes-Switchable Molecular Filters, ***G.V. Rama Rao***, *S. Balamurugan, G.P. Lopez,* The University of New Mexico*; D. Meyer, A. Chilkoti,* Duke University

Elastin-like polypetides (ELPs) are a class of synthetic polypetides comprising of Val-Pro-Gly-Xaa-Gly where Xaa is any amino acid with the exception of proline and exhibit inverse solubility temperature behavior in aqueous solutions. They undergo a transition from hydrophilic (extended conformation) to hydrophobic (compacted conformation) at the lower critical solution temperature (LCST). We demonstrate in this study that when ELPs are encapsulated in a silica matrix, the ELPs can act as molecular switches that control the selective permeability of the membranes. The pores resulting from the transition can selectively transport different molecular species depending on their size. Two different ELPs of molecular weights of 60 kDa (ELP1-150) and 13 kDa (ELP4-30) were used for the present study. Silica-ELP membranes were prepared by sol-gel processing on microcon centrifugal filter units with 30,000 -100,000 molecular weight cut-off membranes and on 1 inch diameter of ultrafiltration discs. The LCST of the membranes was established by permeation measurements and static contact angle measurements. Differential scanning calorimetric studies were employed to determine the LCST of bulk gels and found to be 34 and 44°C for ELP1-150 and ELP4-30 respectively. Cycling of the membranes between 25 and 40-45°C indicates that the membranes possessed reversible, variable permeability while maintaining good mechanical stability. Permeation experiments with various molecular weights of poly(ethylene glycol) (PEGs) on centrifugal filters and ultrafiltration membranes clearly demonstrated that these membranes are acting as a molecular switches by being impermeable below the LCST and permeating the lower molecular weights of PEGs and filtering out higher molecular weight PEGs above LCST.

BI-MoP3 The Silanisation of Tantalum Pentoxide for Biosensor Realisation, ***W. Laureyn***, *F. Frederix, A. Campitelli,* IMEC, Belgium*; J.-J. Pireaux,* FUNDP, LISE, Belgium*; G. Maes,* KULeuven, Belgium

Affinity biosensors allow the detection of affinity based interactions between bio-molecules, which occur e.g. in antibody-antigen recognition or DNA hybridisation. The presence of antigens in an analyte can be verified by the binding of these molecules to their complementary antibodies, immobilised onto a biosensor surface. Tantalum pentoxide is considered as a material with unique properties for biosensor realisation, being chemically very stable and attractive from an electronic point of view, owing to its high dielectric constant. In order to realise tailored bio-interfaces; bromoalkyltrichlorosilanes were deposited on Ta@sub2@O@sub5@. The use of chlorosilanes (as opposed to alkylethoxysilane derivatives) leads to the formation of reproducible and close-packed monolayers on the oxide surface. To allow antibody binding, the bromo-functionality was converted into a carboxyl-functionality, via a one step reaction with mercaptoacetic acid. XPS, FT-IR, contact angle goniometry, cyclic voltammetry and impedance measurements were applied for the characterisation of the cleaning of Ta@sub2@O@sub5@, its silanisation and of the demanded surface reaction. The results of this study indicate the success of our approach. Moreover, distinct differences are revealed for (mixed) silane monolayer formation with short or long-chain chlorosilanes, from the liquid or vapour phase. In future work, the use of bromoalkyltrichlorosilanes will be compared to the silanisation of Ta@sub2@O@sub5@ with allylalkyltrichlorosilanes, followed by an oxidation in order to generate carboxyl groups. This procedure will also allow the immobilisation of antibodies on Ta@sub2@O@sub5@.

BI-MoP4 Organization of Multifunctional Co-polymers on Metal Oxide Surfaces for Optical Biosensing Applications, *N.-P. Huang,* ***I. Reviakine***, *S.M. De Paul, M. Textor, N.D. Spencer,* ETH Zürich, Switzerland

A novel polymeric interface that combines the resistance to non-specific protein adsorption conferred to metal oxide surfaces by poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) with the high affinity of the well-known (strept)avidin-biotin system through the use of a biotinylated-PEG-containing copolymer (PLL-g-PEG/PEGbiotin) has recently been introduced by our group for use in biosensing applications. Biosensor chips with high sensitivity and selectivity can be designed with the above approach, making it an attractive surface technology. Optimization of this system for various biosensor applications depends critically on the knowledge of how the polymer film is organized at the metal oxide interface. Therefore, this work focuses on investigating the organization of the co-polymer film by atomic force microscopy (AFM), quartz crystal microbalance with dissipation (QCM-D) and optical waveguide lightmode spectroscopy (OWLS). This combination of spectroscopic and imaging techniques provides an insight into how the co-polymer mixture is organized at the surface and how this organization evolves with time. The effect of streptavidin addition was also investigated and biotinylated liposomes were used as a well-understood model analyte in AFM and QCM studies.

BI-MoP5 Investigation of the Immobilization Process of Peptide Nucleic Acids, ***J.C. Feldner***, *M. Ostrop, O. Friedrichs, G. Gappa, D. Lipinsky, U. Gunst, S. Sohn, H.F. Arlinghaus,* Universität Münster, Germany

In order to immobilize peptide nucleic acid (PNA) onto Au and Ag coated surfaces, a thiol linker (DTSP, 3,3'-Dithio-bis(propionic acid N-hydroxysuccinimide ester)) was used. The immobilization process of DTSP and PNA to these surfaces can be performed by either binding PNA to DTSP in a solution and then immobilizing it onto the surface or immobilizing DTSP onto the surface and then attaching PNA to it. In both methods, PNA binds to the reactive end group of DTSP and the thiol group of DTSP binds to the Au or Ag surface. The reactive end groups of the DTSP layer can be inactivated using primary amines after immobilization of PNA. Deprotenated (M-H)@super-@ signals of the different PNA bases as well as characteristic peaks of DTSP fragments could be used in TOF-SIMS and TP-SIMS (temperature programmed SIMS) measurements to study and optimize the different immobilization processes. A detailed investigation of the concentration of DTSP and its immobilization time on Au and Ag surfaces showed that the best result could be achieved at a concentration of 10 mM and an immobilization time of 24 hr. The binding of PNA to the DTSP layer takes significantly longer than attaching DTSP to the surface. TP-SIMS data, which are very sensitive to bonding strength, showed that characteristic ion signals of the bases start to decrease at a temperature of about 150°C, with differences in the point of onset for the different bases. From the obtained data it can be concluded that the second attachment method described above is preferable to the first one and also has the advantage of allowing to inactivate the complete Au or Ag surface for unspecific DNA attachment.

BI-MoP6 Biotin-reactive Surfaces Based on @OMEGA@-substituted Alkanethiols on Au(111), ***H. Tran***, *M. Chen, H. Lu, A. Neurauter, S. McManus-Munoz, D. Quincy, T. Langenbacher, P. Peluso, P. Kernen, S. Nock, P. Wagner,* Zyomyx, Inc.

(Oligo)ethylene glycol-containing alkanethiols with @omega@-substituted N-hydroxysuccinimide-ester groups self-assemble in ordered monolayers on Au(111) surfaces (NHS-SAM) and form highly reproducible reactive interfaces for in-situ transformations and biomolecular immobilization. Surface sensitive spectroscopic techniques including FTIRRAS, and XPS, indicate that amino-biotin can be successfully coupled in-situ to the NHS-SAM. Such reactive surfaces have been tested for homogeneous surface coverage and selective binding of streptavidin- and biotin-conjugated dyes and proteins using fluorimetry, radiometry and surface plasmon resonance. These binding data on biotin-functionalized SAMs are compared with binding efficiencies of electrostatically adsorbed biotin derivatized poly(L-lysine)-grafted poly(ethylene glycol) layers on Au(111) surfaces. We incorporated these bioreactive interfaces into microfabricated three-dimensional structures in silicon and used these to test an immunoassay in a microarray format.

BI-MoP7 Reactivities and Biomolecular Immobilization on Self-assembled Alkanethiols with @OMEGA@-substituted N-hydroxysuccinimide-ester Groups on Au(111), ***S. McManus-Munoz***, *D. Martin, C.E.J. Dentinger, R.L. Cicero, H. Tran, M. Chen, P. Kernen, P. Wagner,* Zyomyx, Inc.

Alkanethiols with @omega@-substituted N-hydroxysuccinimide ester groups have been self-assembled on Au(111) surfaces. Stability and reactivity of the functional group were studied using reflection absorption infrared spectroscopy, contact angle, ellipsometry and radiometry measurements. Increased NHS-reactivity was observed for alkanethiols containing (oligo)ethylene glycol units. Effective NHS quenching combined with some effects on non-specific protein adsorption was found with various amino-containing compounds, e.g. glycine and amino-PEGs. Transformation of the NHS-group into a biotin exposing surface was obtained by in-situ coupling of amino-biotin. Specificity and homogeneity of streptavidin and biotin-conjugated protein binding was tested by spectroscopic and microscopic techniques and compared with the binding

efficiencies on physisorbed polymer layers of biotin derivatized poly(L-lysine)-grafted poly(ethylene glycol) on metal oxides and on Au(111).

BI-MoP8 Measuring Bound Water in Protein-resistant Coatings: A Combined OWLS and QCM-D Study of Poly(L-lysine)-g-poly(ethylene glycol), ***S.M. De Paul***, *J. Vörös, I. Reviakine,* ETH Zürich, Switzerland*; C. Galli, M. Collaud Coen,* University of Fribourg, Switzerland*; M. Textor, N.D. Spencer,* ETH Zürich, Switzerland

Metal oxide surfaces coated with poly(L-lysine)-g-poly(ethylene glycol) (PLL-g-PEG) have been shown to resist non-specific adsorption of proteins. The hydrophilicity of the PEG chains is believed to play a crucial role in such behavior. In order to determine the amount of water coupled to PLL-g-PEG at aqueous metal oxide interfaces, we use results from two in situ methods: optical waveguide lightmode spectroscopy (OWLS), which detects the mass of polymer adsorbed at the surface, and quartz crystal microbalance with dissipation (QCM-D), which is sensitive to the mass of the polymer plus the mass of trapped or hydrodynamically coupled water. Complementary information about water content is provided by solid-state NMR measurements. We also examine how the amount of adsorbed polymer and its protein resistance vary with the choice of substrate (e.g., TiO@sub 2@, SiO@sub 2@) and with the surface topography as determined by atomic force microscopy (AFM).

BI-MoP9 Examination of Bacterial and Protein Attachment and Release Using Tunable Poly(N-Isopropylacrylamide) as a Switchable Hydrophobic/Hydrophilic Substratum, ***L.K. Ista***, *S. Mendez, S. Balamurugan, G.P. Lopez,* The University of New Mexico

Poly(N-isopropylacrylamide), or PNIPAAM, undergoes a critical solubility transition in response to temperature. Below 32°C, the polymer is freely water soluble, whereas above this temperature it is insoluble in water. When PNIPAAM is immobilized this property translates to an increase in surface hydrophobicity above the transition temperature (T@sub t@). The process is fully reversible, with restoration of the original degree of solubility or hydrophilicity occurring upon cooling to temperatures below T@sub t@. This switchable characteristic of grafted PNIPAAM has been exploited in the creation of fouling release surfaces. It has been discovered that when thin layers of PNIPAAM are grown from initiator-derivatized. alkanethiolate self-assembled monolayers (SAMs), the range of wettabilities observed above and below T@sub t@ can be tuned by altering the hydrophobicity of the underlying SAM. Because the hydrophobic/hydrophilic transition happens in situ, and rapidly over a short temperature range, tunable PNIPAAM substrata are ideal for examining the effect of changing surface hydrophobicity on the attachment and detachment of biofilm components, i.e. microbes and proteins. We report here the results of attachment and detachment studies on patterned tunable PNIPAAM surfaces using two bacteria which normally exhibit different and opposite responses to subtratum hydrophobicity (Halomonas marina and Staphylococcus epidermidis) as well as studies on adsorption and desorption of a variety of proteins known to play a role in biofilm formation.

BI-MoP10 Novel Immunosensor Interfaces Based on Mixed Self-assembled Monolayers of Thiols, ***F. Frederix***, *W. Laureyn, K. Bonroy,* IMEC, Belgium*; W. Dehaen, G. Maes,* KULeuven, Belgium

An ideal biosensor is characterized by its stability, reproducibility sensitivity and specificity towards a desired analyt. However, reduction of the size of the transducer and thus of the active area, requires an optimization of the sensing area. Our research is therefore also focussing on the biological recognition layer, which is based on an optimized covalent coupling of the antibodies to mixed self-assembled monolayers of thiols on gold. Since cleanliness and structural properties of the gold are of the utmost importance for perfect SAM formation, we have evaluated different cleaning procedures and induced the gold(111) structure. Characterization was performed with XRD and STM. The stability of the SAMs on gold with various properties was evaluated. To attach antibodies and/or avoid non-specific adsorption, novel thiols were synthesized. For standard covalent coupling procedures mercaptoethanol and ethanolamine are normally used as blocking molecules. We have synthesized blocking molecules based on ethylene oxide groups which show enhanced properties towards non-specific adsorption. The mixed monolayer formation was characterized using contact angle, cyclic voltammetry, impedance spectroscopy, XPS and GA-FTIR. The advantages of an orientated immobilization of chemically modified antibodies are demonstrated using SPR. We have evaluated the random amino and streptavidine-biotin coupling in comparison with the orientated aldehyde and thiol coupling procedures. Also the chemical modifications of these antibodies were optimized towards an increased sensitivity. Finally, we compared the sensitivity and selectivity with commercially available biological recognition layers, illustrating their enormous potential for further sensing applications.

BI-MoP11 Piezoresistive Microcantilever Sensors for the Detection of Biological Molecules, ***T.L. Porter***, *M.P. Eastman, D.L. Pace, T.R. Dillingham,* Northern Arizona University

Microsensors capable of recognizing single biological molecules have been fabricated using piezoresistive microcantilever technology. Using 25 base single strand DNA layers as the active sensing material, small piezoresistive microcantilevers in contact with this surface were able to recognize the presence of the complimentary strand, while ingoring the presence of strands differing by 2-5 base units. The analyte recognition is by means of a simple resistance change in the microcantilever, meaning only simple, inexpensive electronics are required for this device. Several sensors may be grouped together to form small bio-sensing arrays.

BI-MoP12 Protein Template-Imprinting to Enhance Specific Protein Adsorption and Cell Adhesion, ***J. Wang***, *X.H. Cheng, J. Schwartz, B.D. Ratner,* University of Washington

Molecularly imprinted polymers (MIPs) are synthetic materials that possess specific recognition properties, and have found applications in enantiomer separation, enzyme mimics, and biomimetic biosensors. Among the common methods for molecular imprinting, the self-assembly approach is important for its resemblance to antibody-antigen, substrate-receptor, and enzyme-inhibitor interactions.@footnote 1@ The key for MIPs to perform recognition is that both the template and imprint are complementary in size, shape, and chemical functionality at the binding site. Nevertheless the self-assembly approach to molecular imprinting has been primarily done in organic solutions, and is less successful in directly imprinting larger biomolecules (proteins). Shi et al.@footnote 2@ developed an alternative approach to imprint protein molecules with disaccharide molecules and polymeric thin films. The protein imprints can preferentially recognize the original template protein, which is mainly attributed to cooperative non-covalent interactions including hydrogen bonds, hydrophobic interactions and van der Waals forces. We applied a similar imprint process with optimized conditions to create highly reproducible albumin (Alb) and fibronectin (FN) imprint surfaces. Surface analyses including ESCA, SIMS, and AFM were performed to characterize the variation in chemical composition after each imprint step. BAE cell adhesion studies demonstrated that the FN imprint surface could enhance cell attachment. Results from @super 125@I labeled binary protein competitive adsorption also showed that the FN imprint surface could preferentially adsorb higher amount of FN in a binary protein solution with Alb as the competing protein. @FootnoteText@ @footnote 1@Haupt, K. and Mosbach, K. Trends Biotechnol. (1998) 16: 468-475 @footnote 2@Shi, H., Tsai, W., Garrison, M., Ferrari, S. and Ratner, BD.. Nature. (1999) 398: 593-597.

BI-MoP13 Surface Technologies to Optimize Osteopontin-immobilized Surfaces for Healing Biomaterials, *S.M. Martin,* University of Washington*; **R. Ganapathy**,* University of Washington, U.S.A*; T. Kim,* University of Washington*; L.A. Martinson,* University of Washington, U.S.A*; D. Leach-Scampavia, S.L. Golledge, C. Giachelli, B.D. Ratner,* University of Washington

Our efforts to develop biomaterial surfaces that modulate the healing and inflammatory response focus on immobilizing specific biological triggers of healing onto a bland, relatively non-protein adsorptive surface. This study illustrates the use of surface analysis tools to characterize such surfaces. Osteopontin (OPN) is a protein known to regulate inflammatory responses. Although its precise role is not fully understood, it has been implicated in wound-healing processes. We have thus chosen OPN as the protein to immobilize in these model experiments. ESCA data in our laboratory demonstrated that poly(2-hydroxyethyl methacrylate) (pHEMA) shows low protein adsorption, making it a suitable material for our immobilization studies. In the present study, a technique using carbonyldiimidazole (CDI) was used to immobilize OPN to the polyHEMA surface. We employed several techniques to verify presence of protein on the polyHEMA surface (ESCA, TOF-SIMS, FTIR) and quantified the amount immobilized (ELISA, radioiodination of OPN). ESCA high-resolution N 1s spectra indicated existence of OPN on the surface, and this data was confirmed by TOF-SIMS. Furthermore, data using I-125-labeled OPN showed a dose-response corresponding to the varying amounts of OPN used for the immobilization experiment. Though less accurate than the radiolabel data, the ELISA showed protein amounts in a similar range as well. These findings represent an important first step toward the creation of novel healing materials for biomedical applications and to using modern surface

analytical tools to verify the surface engineering of a biomaterial. Studies funded by UWEB, EEC9529161.

BI-MoP14 Competitive Oligonucleotide Adsorption Equilibria at a Silane-Water Interface, ***A.D. Suseno***, *R.S. Gascon, J.L. DelosReyes, J.E. Forman,* Zyomyx, Inc.

Adsorption of complimentary oligonucleotide sequences to surface bound oligonucleotide probes can produce surface bound duplex structures that are more prone to dissociation than their solution phase counterparts. The stability (as indicated by the observed melting temperature or Tm) can be altered by a number of factors, including probe orientation on the surface and surface bound probe density. A bound orientation that does not allow the probe to fully interact with its complimentary sequence can result in a non-optimal (and thus lower stability) duplex structure. Such effects are expected when immobilized probes are bound through exo-cyclic amines or crosslinking of thymidine residues to the surface. For surface bound probe densities, crowded surfaces that limit the amount of bound target through unfavorable steric and/or electrostatic interactions serve to destabilize the surface bound duplexes. Likewise, surfaces that interact with the immobilized probe, will compete with adsorption of complimentary sequences and can ultimately reduce duplex stability. Yet, despite these potential hindrances to duplex formation, surface bound probes can adsorb complimentary sequences from solutions in which those sequences are present in double stranded form (where the second strand is non-complimentary to the surface bound probes). We will present results from experiments in which covalently immobilized oligonucleotide probes on silanated substrates interact with both single- and double-stranded oligonucleotide target sequences to illustrate consequences of surface immobilization strategy, as well as what effect the introduction of a competing solution phase duplex formation equilibria has on adsortion to the surface bound probes.

Biomaterials
Room 102 - Session BI+MM-TuM

Biomems & Microdevices
Moderator: W. Knoll, Max-Planck-Institut für Polymerforschung, Germany

8:20am **BI+MM-TuM1 Amplification of Biomolecular Interactions into Optical Signals using Liquid Crystals on Nanostructured Surfaces**, ***N.L. Abbott***, *J. Brake,* University of Wisconsin **INVITED**

Anisotropic interactions between thermotropic liquid crystals and surfaces typically cause liquid crystals to be "anchored" in one or more orientations near surfaces. In this talk, we report the use of surface anchoring phenomena involving liquid crystals for the imaging of biomolecular recognition events on surfaces. The approach is based on the observation that anisotropic forces acting between a liquid crystal and an appropriately designed surface can be perturbed by the formation of biological complex es on the surface. The change in structure of the liquid crystal near the surface is communicated deep into the bulk liquid crystal because the orientational correlation lengths of liquid crystals are typically large (micrometers). We report the design o f surfaces with nanometer-scale topography and patterned surface chemistry such that protein molecules, upon binding to ligands hosted on these surfaces, trigger changes in the orientations of 1-20 micrometer-thick films of supported liquid crystals, thu s corresponding to a reorientation of ~100,000-1,000,000 mesogens per protein. Binding-induced changes in the intensity of light transmitted through the liquid crystal are easily seen with the naked eye and can be further amplified by using surfaces desig n ed so t hat protein-ligand recognition causes twisted nematic liquid crystals to untwist. We also use the average gray-scale brightness of the optical appearance of the supported liquid crystal to construct an optical response curve as a function of the amount of bound protein. This approach to detection of ligand-receptor binding does not require labeling of the analyte, does not require the use of a complex apparatus, provides a spatial resolution of micrometers, and is sufficiently simple that it may find use in rapid, direct-read assays performed away from centralized laboratories.

9:00am **BI+MM-TuM3 Micropatterns of Biomolecules on Silicon Hydride Surfaces**, *J. Pipper, U. Fritz, R. Dahint,* ***M. Grunze***, University of Heidelberg, Germany

Biochips yield a high potential for technological progress in the fields of diagnostics, drug discovery and nanotechnology. They are usually fabricated by photo- and softlithographic methods, various printing techniques or the use of micro electrodes. Common substrate materials are glass-, silicon oxide- and gold surfaces. A powerful alternative to these approaches is the photochemically initiated attachment of terminally functionalized 1-alkenes onto silicon hydride surfaces accompanied by Si-C single bond formation. Although the high potential use of silicon microstructures for biosensing applications has been postulated for years, it has not been exploited yet due to a lack of functional groups suitable for the coupling of biological species. Problems in surface derivatization occur as a result of unwanted parallel chemical reactions and a possible fragmentation of the organic compounds during illumination. This dilemma has now been overcome by temporarily masking the chemical functionalities with non-photolabile protective groups. The paper reports on the spatially resolved, photochemical modification of planar and porous silicon hydride surfaces for the immobilization of DNA, proteins and cells. In combination with photoactive compounds, the method of light induced surface derivatization can also be transferred to organic materials.

9:40am **BI+MM-TuM5 Nano-Scale Effects on the Interfacial Fluidity of Organic Films**, ***R.C. Bell***, *M.J. Iedema, K. Wu, J.P. Cowin,* Pacific Northwest National Laboratory

Interfaces cause fluids in nano-scale spaces to behave very differently than in bulk. We are able to spatially resolve this fluidity with 0.1 nm resolution and show how nanometer films of glassy 3-methylpentane (3MP) are much less viscous at the vacuum-in terface than at the 3MP-metal interface using ion mobility to probe the spatially varying flow properties. The amorphous 3MP films are constructed using molecular beam epitaxy on a Pt(111) substrate at low temperatures (<30 K). A 1 eV hydronium (D@sub 3@O @super +@) ion beam gently deposits ions on or into the films (the latter by depositing more 3MP on top of the ions). The ion motion is monitored electrostatically as the film is heated at a rate of 0.2 K/s above the bulk glass transition temperature of 3 MP (77 K). However, the ions begin to move at temperatures as low as 40 K near the vacuum interface, well below the bulk glass transition temperature. The viscosity near the vacuum-interface at 80 K is found to be 12 orders of magnitude lower than that ex pected of a bulk film. Furthermore, the fluidity perturbations were found to persist over 2.5 nm, which was determined by precisely placing the ions at increasing distances from the interfaces and monitoring the effect on the ion's mobility. Computer modeling is employed to further extract information about the nature of these films.

10:00am **BI+MM-TuM6 Interfacial BioMEMS: Bridging the Micro to the Macro**, ***T. Desai***, University of Illinois at Chicago **INVITED**

A great deal of consideration has been given in recent years to the biological uses of micro-electro-mechanical systems (MEMS). However, such devices are not yet found in many clinical settings due to lack of appropriate interfacing between these devices and the biological world. This talk will describe approaches to engineer interfaces that enhance the biocompatibility and functionality of implantable MEMS based devices. First, the surface modification of silicon-based devices on the nanometer and micron scale to ensure device functionality and integration will be described. Such chemical modifications must be incorporated onto silicon substrates to modulate the interfacial response, while at the same time ensuring compatibility with microfabrication and micromachining processing. Secondly, microfabrication techniques that can be used to selectively attach and spatially localize chemical species in order to control interfacial reactions with the body will be discussed. By integrating surface modification protocols with MEMS processing, one can create device surfaces that interact appropriately with multiple populations of cells and the surrounding tissue. The identification of principles for engineering microdevice surfaces will aid in developing therapeutic bioMEMS, lab on a chip platforms, and drug delivery systems that can more effectively interface with the biological world.

10:40am **BI+MM-TuM8 Dynamics of Biomolecular Recognition on Calibrated Beads in Microfluidic Channels**, ***G.P. Lopez***, *T. Buranda, J. Huang,* The University of New Mexico*; V.H. Perez-Luna,* Illinois Institute of Technology*; L.S. Sklar,* The University of New Mexico

We have developed a new approach for the analysis of biomolecular recognition in microfluidic systems. The method is based on real-time detection of biomolecular binding to receptor-bearing microspheres comprising affinity microcolumns. The microcolumn format ensures efficient analyte contact with receptors and rapid mixing. Molecular assemblies on microspheres can be characterized and calibrated using flow cytometric techniques prior to packing. Model assays demonstrated include direct fluorescence methods of quantitatively detecting recognition of model analytes by protein receptors and ligands displayed in well-characterized affinity matrices. We establish a model system for detection of recognition between a monoclonal antibody and the FLAG@super TM@ epitope tag. The assay can detect sub-femtomole quantities of antibody with good signal-to-noise ratio and a large dynamic range spanning nearly four orders of magnitude in analyte concentration. Kinetic and equilibrium constants for the reaction of this receptor-ligand pair are obtained through modeling of kinetic responses of the microcolumn and are consistent with those obtained by flow cytometry. Because of the correlation between kinetic and equilibrium data obtained for the microcolumns, quantitative analysis can be done in minutes, prior to the steady state endpoint of the recognition reaction. The approach has the potential to be generalized to a host of bioaffinity assay methods including analysis of small molecule analytes, protein and nucleic acid complexes, and microsystem-based multi-analyte determinations.

11:00am **BI+MM-TuM9 Microfluidic Patterning of Biopolymer Matrices for Cellular Pattern Integrity**, ***W. Tan***, *T. Desai,* University of Illinois at Chicago

The ability to design and create biologically relevant patterns via microfluidic patterning on surfaces provides new capabilities for cell biology, the production of biosensors and tissue engineering. However, cellular patterns, defined by microfluidic methods, often lose integrity over time due to cell growth and migration immediately upon removal of the PDMS stamp. In this study, biopolymer matrices were used in conjunction with cellular micropatterning to control cell attachment, growth, and long-term maintenance of these patterns. The incorporation of appropriate matrix materials with microfluidic cell patterning methods results in highly compliant patterns of adherent human endothelial cells (HUVECs) and fibroblasts after several day in vitro. Furthermore, cell type and chemical components in these biopolymer matrices influence the ability of the biopolymer matrices to control cell growth, proliferation and compliance to the patterns. Cell growth and migration in micropatterned biopolymers

such as agarose, collagen, collagen-GAG mimics, and collagen-fibronectin are quantitatively measured and compared, and cell-matrix interactions are also examined over time. Results suggest that the use of an appropriate biopolymer matrix helps to control cell growth and maintain pattern integrity for long periods of time. This is essential for conducting stable biological experiments, as well as achieving control over tissue engineering constructs with multiple cell types.

11:20am **BI+MM-TuM10 High Throughput Techniques for Non Invasive Cancer Cell Detection**, *W.C. Wilson, L.F. Pardo, X.Z. Yu,* ***T. Boland,*** Clemson University

The usefulness of patterned surfaces, which specifically bind antagonists has been recognized for a wide variety of biomedical applications ranging from drug screening to tissue engineering. Current technologies for creating patterned surfaces suffer from many drawbacks. For optimized results, technologies that are flexible, use a large number of different proteins, high-throughput and inexpensive are warranted. Ink jet technology has shown promise in meeting these criteria and commercial systems are being developed. High throughput and quantitative assaying of the patterns is equally challenging. For example, in early cancer detection, it is desirable to detect a few abnormal cells within millions of normal cells. It is unlikely that PCR based techniques or gene chips will be economically feasible tools for early detection since most of the cost will be associated with analyzing normal DNA. Economical high-throughput screening and concentration technologies may be able to discriminate and select abnormal cells for further analysis. We developed a piezo driven protein and cell printer in our laboratory, able to simultaneously deposit picoliter drops of cell or protein solutions out of nine nozzles. The printer can deliver a single cell per drop to a surface with submicron resolution. Furthermore, it is equipped with a robotic arm and conveyer belt allowing for truly high-throughput printing. Examples of its use including for anti angiogenesis drug screening will be presented. Quantitative assaying is done using a cell scanner. The cell scanner has a resolution of less than 2 Âμ, is fully computer controlled, high-throughput and an economically attractive when compared to epifluorescent microscopes. Results will be presented with fluorescently labeled cells demonstrating the potential of the cell scanner for high-throughput discrimination and selection of prostate cancer cells.

11:40am **BI+MM-TuM11 Electrochemically-Activated Switching of Surface Chemistry Using Tethered Molecular Machines**, ***B.C. Bunker,*** *D.L. Huber, J.G. Kushmerick, M. Kelly, C.M. Matzke,* Sandia National Laboratories*; J.F. Stoddart, J. Cao, J.O. Jeppesen, J. Perkins,* University of California, Los Angeles

Sandia National Laboratories is integrating "smart" coatings into microanalytical systems for transporting, separating, and detecting species such as proteins. This paper describes the first demonstration of the use of electrochemically-activated molecular machines to switch surface chemistries. The "motor" for the machines being studied consists of an open aromatic ring system (cyclobis(paraquat-p-phenylene)) referred to as the "blue-box" due to its strong optical absorption properties. Reversible oxidation or reduction of the blue box makes it attract or repel aromatic threads such as functionalized naphthalenes or tetrathiafulvalene (TTF). Researchers at UCLA have succeeded in attaching a disulfide-terminated tail to the blue box which is used to tether the blue box to gold surfaces. Ellipsometry and atomic force microscopy measurements indicate that monolayer films of the blue box are produced. Electrochemical measurements indicate that while the voltages required to reduce the blue box are similar to voltages known to induce switching of the box in solution, adsorption of naphthalene threads is irreversible. Reversible switching is only seen for TTF threads that can themselves be oxidized. Contact angle measurements show that reversible changes in surface chemistry can be induced using appropriate threads. A simple microelectronic device has been constructed to demonstrate how the molecular machines can be used to move liquids or dissolved species within microfluidic systems.

Surface Science
Room 120 - Session SS+BI-TuM

Poirier Memorial Session: Self-Assembled Monolayers I

Moderator: N.D. Shinn, Sandia National Laboratories

8:20am **SS+BI-TuM1 Greg Poirier's STM Landscapes of Alkanethiol Monolayers on Gold: A Retrospective**, ***M.J. Tarlov,*** National Institute of Standards and Technology **INVITED**

The surface science community lost one of its bright young stars, Gregory E. Poirier, who passed away in September at the age of 39. During Greg's brief career at NIST he made many noteworthy contributions in the areas of surface science and chemical sensing, however, it was his STM studies of alkanethiol self-assembled monolayers (SAMs) on gold that earned him the greatest recognition. Greg's STM images were some of the first to reveal the structural complexity and phase behavior that governs the 2-D world of alkanethiols on gold. Through meticulous and rigorous interpretation of these images he unlocked many structural details of SAMs and gained an understanding of the molecular forces that govern the assembly of SAMs. This talk will review some of the highlights of Greg's STM studies including the rich variety of molecular-scale crystal structures of alkanethiol SAMs, their associated defect structures, and the development of a general mechanism for SAM formation.

9:00am **SS+BI-TuM3 Imaging and Diffraction: Two Complementary Probes of Self-Assembled Monolayer Structure and Properties**, ***G. Scoles,*** Princeton University **INVITED**

Using Self-Assembled Monolayers (SAMs) as test systems, a few introductory examples of the well known complementarity between imaging (local SAM structure and defects) and diffraction (precise determination of overall SAM structure) will be given. Starting from the pioneering detection, by Poirier, of the c(4x2) superlattice superimposed to the basic hexagonal structure of alkylthiol SAMs on Au(111), we will review the present status of the question concerning the presence of at least two inequivalent sulfur atoms in the equilibrated monolayers. After reviewing the overwhelming experimental evidence that points in that direction, we will present recent Density Functional Theory calculations that may finally provide a way out of this ten year old controversy. Returning to the complementarity between imaging and diffraction, we will point out a few less known features of both methods. In particular, the sensitivity of atomic beam diffraction to very small vertical displacements (not detectable with either X-rays or STM) will be discussed along with the possibility, offered by X-rays, to provide information on buried SAM interfaces. We will conclude showing how the synthetic flexibility afforded by SAMs coupled with the local sensitivity of STM provides unique opportunities to understand electron transfer processes at the organic/metal interface.

9:40am **SS+BI-TuM5 Characterization of Self-assembled Monolayers Using Near-edge X-ray Absorption Spectroscopy**, ***T.M. Willey,*** Univ. of California at Davis and Lawrence Livermore National Lab*; A.L. Vance, A.W. vanBuuren,* Lawrence Livermore National Lab*; C.F.O. Bostedt,* Univ. of Hamburg, Germany, and Lawrence Livermore National Lab*; G.A. Fox, A.J. Nelson, L.J. Terminello,* Lawrence Livermore National Lab*; C.S. Fadley,* Unive. of California at Davis and Lawrence Berkeley National Lab

We have investigated methyl and carboxyl terminated alkanethiols (hexadecanethiol and mercaptohexadecanoic acid) and other more complex self-assembled monolayers (SAMs) on Au(111). We characterize these SAMs using x-ray absorption at the Carbon K-edge, Sulfur L-edge, ans well as Oxygen and Nitrogen K-edges where applicable. Near-edge X-ray Absorption Spectroscopy (NEXAFS) gives information about chemical state, and polarization effects in these spectra probe the orientation of chemical bonds. Simple, carboxyl-terminated alkanethiols supposedly do not form well-ordered films. We currently investigate attachment, uniformity, and order of these films as a function of solvent, chemical state of the carboxyl group, and by intermixing with well-ordering methyl-terminated alkanethiols. We have also investigated attachment and order of more complex, disulfide containing thioctic acid derivatives. Preliminary results indicate that we have been successful in chemisorbing both sulfur-functionalized ends of a number of these molecules to the gold surface. T. Willey acknowledges a Student-Employee Graduate Research Fellowship through LLNL. This work is supported by the U.S. Department of Energy, BES Materials Sciences under contract W-7405-ENG-48, LLNL.

10:00am **SS+BI-TuM6 Structure of Alkyl Thiol and Dialkyl Disulfide on Au(111)**, ***H. Nozoye***, Nanotechnology Research Institute, AIST, Japan; *T Hayashi, C. Kodama,* University of Tsukuba, Japan; *Y. Morikawa,* JRCAT, AIST, Japan

The structure and formation process of self-assembled monolayers on Au(111) have been a long-standing unsolved problem. We determined the adsorption state of alkane thiol and dialkyl disulfide with different alkyl chain length on Au(111), which are prototypical self-assembled monolayer systems, by using temperature programmed desorption (TPD), high sensitivity low energy electron diffraction (LEED), high resolution electron energy loss spectroscopy (HREELS) and density functional theory (DFT) calculation. We concluded that the SH bond of alkane thiol and the SS bond of dialkyl disulfide break below room temperature, spontaneously desorbing hydrogen and resulting in the formation of highly ordered adsorption state of thiolate on the surface. The spectra above 500cm@super-1@ contain the information of the orientation of the alkyl moiety, however, those of low energy region contain the information of the bonding between S and Au. HREELS spectra of low energy region were almost the same for all the thiolate species, irrespective of the difference of the coverage of the thiolate and of the chain length of alkyl moiety, although the spectra above 500cm@super-1@ were changed depending on the coverage of the thiolate species. The change of the HREELS spectra above 500cm@super-1@ agreed with the conclusion obtained from STM measurements, that is the lying-down configuration in the low coverage region and standing-up configuration at the saturation coverage. The HREELS spectra of the low energy region were analyzed with the DFT calculation; The location of S of the thiolate on Au(111) is the bridge site and the SC bond is inclined about 50 degree from the surface normal. From these results we concluded that the structure of the root part of the thiolate species is common, at least for alkyl thiolate with relatively short chain, notwithstanding the difference of the orientation of the alkyl moiety, that is the lying-down and standing-up structures.

10:20am **SS+BI-TuM7 Two-dimensional Phase Diagram of Decanethiol on Au (111)**, ***J.M. White***, *W.P. Fitts,* The University of Texas at Austin **INVITED**

Based on variable temperature ultrahigh vacuum scanning tunneling microscopy data, we propose a two-dimensional phase diagram of monolayer decanethiol on Au(111). Four triple point temperatures were determined: T@sub1@ at ~ 27 @degree@C, T@sub 2@ at ~ 33 @degree@C, T@sub3@ at ~ 35 @degree@C and T@sub 4@ ~ 56@degree C. T@sub 1@ defines the lowest temperature melting point and T@sub 4@ defines the temperature above which striped phases are metastable. These data provide a fundamental framework to understand and control meso-scale monolayer structure; moreover, they provide fundamental insight into two dimensional phase behavior of molecules with many degrees-of-freedom.

11:00am **SS+BI-TuM9 Odd-Even Effects in the Electron-induced Damage of Biphenyl-substituted Alkanethiol Self-assembled Monolayers**, ***M. Zharnikov***, *S. Frey, H.-T. Rong,* Universität Heidelberg, Germany; *M. Buck,* University of St Andrews, U.K., Scotland; *K. Heister, M. Grunze,* Universität Heidelberg, Germany

Self-assembled monolayers (SAMs) formed on Au(111) from biphenyl-substituted alkanethiols CH@sub 3@(C@sub 6@H@sub 4@)@sub2@(CH@sub 2@)@sub n@SH (BPn) exhibit odd-even changes in the packing density and the tilt angle of the biphenyl moieties with varying length of the aliphatic part. We have applied X-ray photoelectron spectroscopy, near-edge X-ray absorption fine structure spectroscopy, infrared absorption spectroscopy, and advancing contact angle measurements to study electron beam induced damage in these systems as well as in pure aromatic biphenylthiol (BP0) SAMs on Au substrates. Although the character of the electron-induced damage in all investigated SAMs was found to be similar, the extent and rate of the observed changes exhibited a clear correlation with the packing density and orientation of the biphenyl moieties in the BPn SAMs: The densely packed BPn films are noticeably more insensitive (in terms of the orientational order, irradiation-induced desorption, and anchoring to the substrate) towards electron irradiation than the loosely packed BPn layers. The extent of the irradiation-induced damage in pure aromatic BP0 SAMs was observed to be very close to that in the loosely packed BPn films, which indicates that the former films are in some sense also "loosely packed". Considering the potential applications of the aromatic SAMs as lithographic resist or template, the introduction of the short aliphatic chain in the respective molecules provides a simple and efficient way to manipulate their reaction toward ionizing irradiation in a desirable way. This work has been supported by the German BMBF (05 SF8VHA 1).

11:20am **SS+BI-TuM10 Highly Ordered Organic Monolayers with Reduced Antiphase Domain Walls Due to Growth from Two-dimensional Gas- and Fluid- Phases**, ***L. Gross***, *C. Seidel, H. Fuchs,* University of Münster, Germany

The growth process of monolayers of perylene and coronene on the metal surfaces Ag(110), Au(110) 1x2 and Au(111) 23x@sr@3 was investigated by means of low energy electron diffraction (LEED) and scanning tunneling microscopy (STM). The preparation was done by molecular beam epitaxy (MBE), with the possibility of LEED or STM measurements during evaporation. Both molecules show (restricted on Au(110) 1x2) an evolution from isotropic disordered structures in the submonolayer regime to a highly (substrate-dependent) ordered monolayer. In all presented systems the adsobate begins to form highly ordered structures just before the coverage of a complete monolayer is reached. The final monolayer structures are coincident, except coronene on Au(111) which is commensurate, furthermore perylene on Au(110) and coronene on Au(111) show uniform domain orientation. The lateral ordering process of these molecules allows epitaxial growth with a reduced density of antiphase domain boundaries, because crystallization does not start from islands in the submonolayer regime, but from two-dimensional gas- and fluid- phases.

Biomaterials
Room 102 - Session BI-TuA

Non Fouling Surfaces and Theoretical Concepts
Moderator: H.J. Griesser, CSIRO, Australia

2:00pm **BI-TuA1 Surface Forces and Coating Properties Involved in Protein Repellency**, *P. Kingshott, H. Thissen, L. Meagher, P. Hamilton-Brown,* ***H.J. Griesser,*** CSIRO, Molecular Science, Australia **INVITED**

PEO coatings have attracted much interest as non-fouling coatings. However, literature data show varying extents of reduction in protein adsorption with PEO coatings prepared in various ways, and use of techniques that may not always have been sufficiently sensitive to support claims of non-fouling (as opposed to low-fouling). We have immobilized PEG chains of different lengths onto surfaces with different densities of pinning groups and at room temperature as well as under cloud point conditions to study how these parameters affects macromolecular conformations and protein resistance. We have also investigated the limits of detection of adsorbed proteins on the 'best' coatings by the sensitive surface analytical methos XPS, ToF-SSIMS and MALDI. Cell colonization was found to be totally inhibited and this could be attributed to the inability of fibronectin and vitronectin to adsorb to the coating. Using a laser ablation technique, patterns were then created of cell-adhesive islands within a PEO-coated surface area. It was shown that the cells recognized edges with high precision. Finally, surface force curves were acquired using a colloid-modified AFM tip in order to probe for the interfacial forces that contribute to incomplete or complete protein repellency. Our PEO coatings differ markedly in structure and some properties from oligo-EO coatings prepared by SAM methodology, yet give analogous results in terms of resistance to fouling. Based on this and data with polysaccharides (Hartley et al, this meeting) we speculate that protein resistance does not require a 'magic' chemistry or a fully extended 'brush' structure; a highly hydrated coating that possesses a repulsive surface force due to steric-entropic-osmotic effects on compression, of sufficient magnitude and range to screen attractive van der Waals and electrostatic forces emanating from the substrate, is sufficient. The chemical composition may not matter as long as the coating is well hydrated and of a minimal thickness, and protein repellency may solely be a result of appropriate physico-chemical properties. Moreover, charge neutrality is required, as negatively charged coatings such as hyaluronan are effective only against some fouling situations. For insance our PEO coatings are neutral and screen substrate charges, thus repelling proteins of both charge signs.

2:40pm **BI-TuA3 Water-Uptake of Poly(ethylene glycol)-terminated Self-Assembled Monolayers during Film Formation**, ***J. Fick,*** *S. Tokumitsu, M. Himmelhaus, M. Grunze,* Universität Heidelberg, Germany

Self-assembled monolayers (SAMs) terminated by poly(ethylene glycol) (PEG; MW = 2000 Dalton) formed on polycrystalline gold have proven to provide an interesting model system for the study of grafted PEG chains with different morphologies, such as mushroom, polymer brush, and crystalline-like phase. The desired structure can be obtained simply by varying the immersion time of the substrate in solution, because the adsorbed molecules adopt the respective conformations as a function of coverage. As there is still a controversial discussion about the origin of the unique properties of PEG in terms of protein resistance and the roles that both, morphology of the PEG and bound water molecules might play, we have studied the water-uptake of the PEG-SAMs as a function of surface coverage by optical spectroscopies. The SAMs were adsorbed from solvent mixtures with distinct amounts of water added. We present the dependency of the adsorption kinetics as a function of various parameters, such as polarity, water-content of the solvent, and temperature during adsorption.

3:20pm **BI-TuA5 Novel PEO-containing Copolymers as Protein Repellent Additives In Polyurethanes: Evaluation of Protein Interactions by Radiolabelling, XPS and MALDI**, *J.H. Tan,* McMaster University, Canada*;* ***K.M. McLean,*** *T.R. Gengenbach, H.J. Griesser,* CSIRO Molecular Science, Australia*; J.L. Brash,* McMaster University, Canada

Polyurethanes (PUs) have long been used for medical applications, mainly because of their excellent mechanical properties. However, there is a need to improve the biocompatibility of these materials. Polyethylene oxide (PEO) has gained recognition as a biocompatible material and appears to interact minimally with proteins and cells. In this work, materials have been developed based on PEO-containing additives that can be applied to conventional PUs. The additives are amphiphilic triblock copolymers, PEO-PU-PEO, the middle segment of which has the same structure as the PU substrate. We hypothesize that such additives should interact strongly and be compatible with any polyurethane of structure similar to the middle segment, and that they should migrate to the PU-aqueous interface. Copolymers were synthesized using PEO blocks of varying MW (550, 2000, 5000) and a central PU block of MW 5000. Materials were prepared by blending the block copolymers with a base PU. The surfaces were characterized by water contact angle and XPS. Adsorption of proteins was investigated by radiolabelling and by XPS. The water contact angle data showed that the blends became more hydrophilic with increasing copolymer content. Radiolabelled fibrinogen expts showed that adsorption was much lower on the blends than on the unmodified PU, in some cases showing reductions of greater than 99%. For the 10% blends, surprisingly, adsorption decreased in the order PEO5000>2000>550. This "inverse dependence" is attributed to slower diffusion of the higher MW copolymers to the interface. The protein adsorption characteristics were also investigated using XPS and surface-MALDI using a range of individual plasma proteins (fibronectin, vitronectin, albumin, insulin, fibrinogen and IgG) and whole plasma. XPS results confirmed that protein adsorption on the blends was negligible compared to the unmodified PU. Ongoing surface MALDI experiments also indicate low adsorption on the copolymer-PU blends.

3:40pm **BI-TuA6 Surface Modification of Poly(Vinyl Chloride) Intubation Tubes to Control Bacterial Adhesion**, ***D.J. Balazs,*** *Y. Chevolot, K. Triandafillu, H. Harms, C. Hollenstein, H.J. Mathieu,* Swiss Federal Institute of Technology - Lausanne, Switzerland

Bacterial colonization of intubation tubes is responsible for 30% of all nosocomial pneumonia cases, 40 % of which lead to death, despite aggressive antibiotic therapy.@footnote1@ Therefore, a strategy to reduce bacterial adhesion is desirable. We are developing an approach based on the surface modification of the polymer used for this application, medical grade poly(vinyl chloride) (PVC). The strategy is to mask the PVC substrate with a chemically inert teflon-like fluoropolymer layer, which serves as an ideal platform for further surface modification due to its low surface energy.@footnote 2@ Protein@footnote 3@ and bacterial@footnote 4@ repellant molecules, e.g. amphiphilic Pluronics@super R@, are bound to the fluoropolymer films using hydrophobic-hydrophobic interactions. This paper investigates fluoropolymer films created on PVC substrates through plasma-enhanced chemical vapor deposition. The films are deposited in an RF-plasma reactor, using C@sub 2@F@sub 6@ as a precursor and H@sub 2@ as a carrier gas. XPS data suggest that the films completely mask the substrate, as no remaining signatures of PVC are detectable. Moreover, alpha step measurements show a uniform film, with a thickness of approx. 200 nm. The fluoropolymer films were found to be highly hydrophobic, with a water contact angle > 100 °. Preliminary contact angle measurements of the Pluronic@super R@ surfaces show a significant decrease in contact angle, (approx. 20 °) indicating adhesion to the fluoropolymer layer. Feedback from imaging XPS is then used to optimize Pluronic@super R@ monolayer formation on the fluoropolymer film. Protein adsorption and in vitro bacterial adhesion studies will also be reported. @FootnoteText@ @footnote 1@ J. L. Vincent et al (1995) JAMA 274: 639-644 @footnote 2@ I. Noh et al (1997) J Polym Sci Pol Chem 35: 1499-1514 @footnote 3@ M. Paulsson et al (1993) Biomaterials 14: 845-853 @footnote 4@ M. J. Bridgett et al (1992) Biomaterials 13: 411-416.

4:00pm **BI-TuA7 XPS-Mediated Robust Design Used to Optimize Hyaluronic Acid Surface Immobilization**, ***T.A. Barber,*** University of California, Berkeley*; R.A. Stile,* Northwestern University*; D.G. Castner,* University of Washington*; K.E. Healy,* University of California, Berkeley, U.S.A

A major limitation of scaffold-based cartilage tissue engineering approaches is the inability of the delivery scaffolds to adhere to the tissue lining cartilaginous defects. Previously, thermo-responsive P(NIPAAm-co-AAc) hydrogels were engineered to support chondrocyte viability and promote articular cartilage-like tissue formation in vitro. An objective of the current research is to functionalize these hydrogels with bioactive peptides to support specific interactions with components found in cartilaginous extracellular matrix (specifically Hyaluronic Acid (HA)). It is hypothesized that these interactions will significantly enhance scaffold-defect adhesion. A model HA surface was developed to test this hypothesis by quantifying these interactions. A commonly used strategy for HA surface immobilization exploits a carbodiimide reaction between the carboxylic acid groups present in HA, and an aminofunctional surface. However, a wide variety of reaction conditions have been reported, and it is unclear

which elements are critical to this HA-grafting approach. Consequently, Robust Design methods were employed to optimize the HA-grafting procedure on aminofunctional glass substrates. First, a 4-factor, 3-level orthogonal array (L@sub9@) was constructed to monitor the effects of HA concentration, coupling buffer (CB), CB pH, and Carbodiimide/N-Hydroxysulfo-succinimide concentration ([EDC/NHS]) on HA-grafting success. XPS C/Si ratios were used to assess factor effects. Deconvolution of the L@sub9@ identified HA concentration, CB, and [EDC/NHS] as the dominant process variables. Subsequently, a 3-factor, 2-level orthogonal array (L@sub4@) was used to further refine the HA-grafting conditions. Evaluation of the L@sub4@ suggested optimal levels for HA concentration (2.5μM), CB (10mM HEPES), and [EDC/NHS] (100mM/25mM). Optimal grafting conditions will be utilized for preparing model surfaces to evaluate the adhesive properties of the functionalized gels using JKR adhesion testing.

5:00pm **BI-TuA10 Theoretical Prediction of the Enthalpic and Entropic Contributions of the Change in Gibbs Free Energy for Peptide Residue Adsorption onto Functionalized SAM Surfaces**, ***R.A. Latour,*** Clemson University

The thermodynamic energy contributions of the change in enthalpy (dH) and entropy (dS), and their summation to calculate the change in Gibbs free energy (dG), provide a very useful tool to predict complex biomolecular behavior. This approach has been successfully applied to address a wide range of biomolecular problems such as the prediction of protein and RNA folding and ligand-receptor binding for rational drug design. A similar approach holds great potential to be applied to understand and predict the adsorption behavior of proteins to synthetic surfaces. A protein is composed of specific sequences of peptide residues arranged in a well-defined structural organization. Protein-surface adsorption can be expressed as a set of intermolecular (residue-surface) and intramolecular (residue-residue) interactions with the minimization of these energetic contributions determining the final conformation and orientation of adsorbed protein. In this study, computation chemistry (MOPAC/PM3/COSMO) was combined with wetting data to predict dH, dS, and dG contributions for the adsorption of individual peptide residues (alanine, serine, lysine) on functionalized SAM surfaces (methyl, hydroxyl, carboxyl) as a function of surface separation distance (SSD). The results are in close agreement with other more generalized continuum-based theories of adsorption and predict how dH and dS from residue/surface and solvent restructuring effects contribute uniquely for each residue/surface pair. These results will serve as the foundational building blocks of more advanced treatments to quantitatively predict protein adsorption behavior with subsequent application for biomaterials surface design.

Biomaterials
Room 134/135 - Session BI-TuP

Surface Characterization and Non-Fouling Surfaces Poster Session

BI-TuP2 Short-term Oxidation of Polymer Films Deposited from Pulsed Radiofrequency Allylamine Plasmas, ***J.D. Whittle***, *G.R. Kinsel, R.B. Timmons,* University of Texas at Arlington

Plasma deposited films are seen as a promising route to the synthesis of novel functional coatings for a large number of potential applications. Allylamine deposited films in particular are of great interest in the biomaterials field as surfaces for protein adsorption. Studies of the long-term aging of these plasma polymers have shown that the oxygen content of the films changes over extended periods of time. Earlier work has shown that the oxygen content of allylamine films deposited from continuous wave plasmas increased from around 2% for a fresh sample, to around 10% after a year of aging in the laboratory, with the greatest change in composition being within the first 48h. In addition, some loss of nitrogen from the films has also been observed. In this study, we concentrate on the changes in chemistry over the first few days, and in particular the first 12 hours following deposition. The surface chemistry is investigated by X-ray photoelectron spectroscopy (XPS) and Matrix Assisted Laser Desorption/Ionization Mass spectrometry (MALDI-MS). Using XPS we investigate the stability of the plasma polymer surfaces in the UHV environment using different substrates for deposition to determine what the source of the oxidative species may be. A small amount of oxygen is always present in these plasma polymers, which may be due to the unavoidable exposure to the atmosphere between completing the deposition, and insertion of the sample into the spectrometer. Further, by analyzing samples exposed to the laboratory atmosphere for specific lengths of time, we show how the surface chemistry evolves in the first few hours following deposition. We also examine the effect of plasma power and pulsing duty cycle on the post-deposition properties of the films.

BI-TuP3 Fast Impedance Spectroscopy Measurements on Supported Lipid Bilayer Membranes with and without Incorporated Ion Channels, ***G. Wiegand***, *S. Beyer, N. Arribas-Layton, P. Wagner,* Zyomyx Inc.

A substantial part of the mammalian proteome is represented by proteins that are either associated or incorporated into lipid bilayer membranes. Our goal is to provide appropriate platform assays and transducer technologies for the functional analysis of membrane proteins. Our special focus is on ion channels due to their pharmacological relevance. Because ionic flux thru an ion channel generates an electrical signal, electronic transducer technologies are the most direct detection method for ion channel analysis. We developed a method of fast impedance spectroscopy that combines the power of a spectroscopic technique providing high information content with the millisecond time resolution of a fast analytical tool. In biophysical experiments, time dependent quantities such as the membrane resistance and the membrane capacity are obtained from the measured sequences of impedance spectra. Supported lipid bilayers provide membrane matrices for protein incorporation that are coupled to solid surfaces. Supported bilayer applications take advantage of the high membrane stability imparted by the solid support, and of the improved accessibility for analytical tools due to the two-dimensional geometry. As a result of the chip compatibility, supported membrane systems are potentially useful in high-throughput technologies. By application of fast impedance spectroscopy, dynamic properties of supported lipid bilayers with and without incorporated ion channels are studied during formation, relaxation and in various states of conduction.

BI-TuP5 Optical Inverted Microscope with a Scanning Near Field Optical Microscope to Study Biological Material, *A. Cricenti,* ***R. Generosi***, *M. Luce, P. Perfetti,* ISM-CNR, Italy

A scanning near field optical microscope (SNOM) has been added to a standard inverted optical microscope with the dedicate aim of characterizing the inner parts of biological molecules. Therefore, in addition to the requirements of reliability and mechanical stability we have carefully looked to analyzing a sample with all available geometries for input/output of photons, in order to get as many information as possible. The SNOM unit consists of a support mounted on the optical microscope arm containing a piezoelectric scanner. The reflectivity of the sample can be measured by applying different methods: the sample can be illuminated on top by an external source, as well as by the optical fiber used for the detection of the reflectivity signal. Absorption experiments can be easily performed by detecting the transmitted signal through the optical apparatus of the inverted microscope.Also fluorescence signal can be simultaneously detected. Reflectivity, transmissivity and fluorescence measurements will be presented on several biological systems, with a resolution well below the diffraction limit.

BI-TuP6 Investigation of Bone Tissues using Infrared Spectroscopic Ellipsometry, ***G.M.W. Kroesen***, *J.-C. Cigal, E. Stoffels, B. van Rietbergen, R. Huiskes,* Eindhoven University of Technology, The Netherlands

Small fractures on the bone surface, called micro-cracks, are formed throughout the lifetime as a result of e.g. mechanical stress. In individuals of advanced age, these defects are no longer efficiently repaired by the organism. Increasing density of micro-crack is one of the important factors which lead to osteoporosis: the severe loss of bone mass and attendant fragility of the skeleton. The size of micro-cracks is in the order of 10 microns, and they are difficult to detect in vivo. Apart from these fractures, the chemical composition of the bone surface is expected to change in the course of ageing. Spectroscopic ellipsometry is a powerful but non-destructive technique of analysing complex surfaces, and it seems very suitable in a study of bone tissues. We developed a spectroscopic ellipsometer combined with a Fourier transform spectrometer in the middle infrared range (wavelength of 2.5 to 10 microns). This device allows to collect accurate data on the chemical composition of the bone surface. In addition, it can provide information about the surface roughness, which is useful in determining the density of micro-cracks. Ellipsometry is a purely physical method, and this novel application to the complex biological environment poses many technical challenges. We will present preliminary results on ellipsometric analysis of bone surfaces, including infrared spectra of several bone samples. In the subsequent study we will investigate how the ageing of the bone tissue is reflected by its infrared properties.

BI-TuP7 Changes in Bone Surface after Exposure to an Electric Discharge, ***J.H.R. Feijen***, *C.Y.M. Maurice, E. Stoffels, G.M.W. Kroesen, B. van Rietbergen, R. Huiskes,* Eindhoven University of Technology, The Netherlands

Human bones are subject to a continuous process of regeneration. Due to mechanical stress, cracks on a microscopic scale are generated in bone tissue, but in the healthy situation these cracks are repaired before they can lead to serious damage. In the case of disturbed bone regeneration, however, due to osteoporosis, drugs that inhibit bone resorption or bone cancer, the mechanical integrity of bone is impaired by accumulation of micro cracks or large metastatic defects. Treatment of bone diseases in vivo is nowadays very difficult. We consider an alternative method of bone surface processing, using non-equilibrium (cold) electric discharges. These plasmas combine high reactivity with non-destructive character. In this study we attempt plasma treatment and observe its impacts on the surface of bone tissues. These impacts are change in roughness, etching of some layers, removal of cells, etc. Since the concept of exposing living tissues to electric discharges is new, the presented results are preliminary and the medical implications are not yet resolved. For this experiment we employ a low-pressure inductively coupled plasma (ICP), supplied with diagnostics. A Langmuir probe, an energy-resolved mass spectrometer and a Doppler shifted laser-induced fluorescence (DSLIF) techniques are used to monitor the parameters of the plasma. With an Environmental Scanning Electron Microscopy (ESEM) we record images of the surface before and after exposure to the plasma. Several gases will be investigated, like oxygen, hydrogen and argon, and plasma treatment under various conditions (varying pressure, power and electric bias) will be performed. In the continuation of this work, cold atmospheric discharges will be used for bone treatment.

BI-TuP8 Glass Ionomer Cements: Probing Uptake from Solution using Surface Sensitive Techniques, *B.M. Hutton, G. Palmer, P.C. Hadley,* University College London, UK*; T.A. Steele, A.J. Eccles,* Millbrook Instruments Ltd., UK*; R.W. Billington, G.J. Pearson,* Queen Mary, UK*;* ***F.H. Jones***, University College London, UK

Glass ionomer cements (GICs) are dental filling materials with the ability to take up ionic species (e.g. F@super -@) from solution and store them within the cement matrix for subsequent re-release. This offers the potential for controlled drug release in-vivo. Previously, ion uptake and release by such materials has been determined primarily by measuring ion concentration in solution.@footnote 1@ Although this approach provides useful data on the concentration of ionic species as a function of time, little is learned about the mechanism of uptake and release. In the current work, X-ray photoelectron spectroscopy (XPS) and secondary ion mass

spectrometry (SIMS) have been used to examine ion uptake. Cements were analysed after the introduction of fluoride either by doping or by immersion in KF(aq) of various concentration. Immersion of a GIC based on poly(acrylic acid) and a calcium aluminosilicate glass was found to result in the formation of a calcium and fluoride rich surface layer, while doping by mixing with KF solution during GIC preparation resulted in no such surface layer. The formation of CaF@sub 2@-like layers on immersion was entirely unexpected on the basis of previous solution-based experiments and may explain differences in measured uptake kinetics. The use of these techniques has been extended to examine the inclusion of molecular active species (amprolium hydrochloride and chlorhexidine acetate) within the GIC matrix. Both species could be detected in GIC samples irrespective of whether they were included by mixing or by immersion in solution. However, relative peak intensities indicated that the binding of the active molecule is dependent on the method of inclusion. Significant applications in the study of the uptake and release mechanisms of active species such as antibacterial and antifungal agents are envisaged. @FootnoteText@ @footnote 1@ Hadley P, Billington RW, Pearson GJ. Biomaterials 1999;20:891-897.

BI-TuP9 Morphological Analysis of the Collagen Structure of Regenerated Rat Tendons Following Laser Photo-stimulation, ***V. Baranauskas,*** Universidade de Campinas - Brazil, Brazil*; N.V. Parizotto,* Universidade Federal de Sao Carlos - Brazil

Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) were used to comparatively study the structure of regenerated rat tendons submitted to laser photo-stimulation after a surgical injury that extracted around 6 mm of Achilles'' tendon (tenectomy) of the left forefoot. The experiment was performed using male Rattus norvegicus albinus, lineage Wistar, with body weights in the range of 250 to 300 g, and 90 days old. The healing course was monitored at 7, 14, 21 or 28 days after the injury. The animals were divided into four groups. One group was used as a control and the other three were subjected to irradiation of doses of 0.5, 5.0 and 50 Jcm-2, respectively. A He-Ne laser of 6 mW power (L = 632.8 nm) was used. AFM and SEM images, at different magnifications, allowed the identification of the time-dependence of the recovery, measured by the organization of the collagen fibers. Comparison of natural recovery processes with the laser photo-stimulation procedures will be discussed.

BI-TuP10 Evolution of a UHV Compatible Heater for TSG Preparation, *M. Hasselblatt,* ***B. Jackson,*** *M. Heidecker, P. Wagner,* Zyomyx, Inc.

Template Stripped Gold (TSG) surfaces have been used extensively as a source for ultra-flat substrates. The preparation of these thin gold films relies on accurate temperature control. Heating in UHV / HV is always difficult since an accurate temperature measurement requires excellent thermal contact with the sample due to the lack of convection. Working with layered crystals like mica makes matters worse. Here we present the evolution of an ultra-high vacuum compatible heating plate that is optimized for the preparation of TSG. Initial versions of this heater were successfully built by casting Omegabond 600 High Temperature Chemical Set Cement into a Teflon mold including pre-coiled Omega Nickel Heating Wire and thermocouple wire. The current design features an exceptional degree of thermal homogeneity over approx. 9 square inches with variations of less than 1% and also a modular design to improve serviceability.

BI-TuP11 Imaging Biomolecules for Skin Cancer Demarcation, ***M.B. Ericson,*** *A. Rosén,* Chalmers University of Technology - Göteborg University, Sweden*; A.-M. Wennberg, C. Sandberg,* Sahlgrenska University Hospital - Göteborg University, Sweden*; F. Gudmundsson,* Chalmers University of Technology - Göteborg University, Sweden*; O. Larkö,* Sahlgrenska University Hospital - Göteborg University, Sweden

Protoporphyrin IX, Pp IX, is a photoactive porphyrin molecule formed in the cell heame synthesis. It has been shown that Pp IX is formed to a larger extent in tumor cells due to enzymatic and metabolic differences compared to normal cells. This effect can be enhanced by exposing the cells to an excess of aminolevulinic acid, ALA, a precursor in the heame synthesis. By imaging the fluorescence from Pp IX molecules in the skin, the extension of skin tumor can be visualised with respect to the enhanced Pp IX production. This technique is based on photodynamic therapy, PDT, which is a new clinical treatment for cancer that has developed over the past 25 years. In a clinical study of 40 patients with basal cell carcinoma, a malignant type of skin cancer, the Pp IX fluorescence was recorded by a CCD camera set-up. The lesions were treated with ALA cream and thereafter the fluorescence was visualised by using filtered mercury lamps as excitation light-source. The contrast in the fluorescence images was evaluated as a function of ALA application time in order to optimise the technique. The study showed a correlation between the fluorescence images and histological pattern however the individual variations were large. Further studies are planned in order to further improve the technique.

BI-TuP12 Characterization of the Crotalus Durissus Terrificus Venom by Atomic Force Microscopy, *V. Baranauskas,* ***J. Zhao,*** Faculdade de Engenharia Eletrica e Computacao - UNICAMP, Brazil*; D.M. Dourado,* UNIDEP - Brazil*; M.A. Cruz-Hofling,* Instituto de Ciencias Biologicas - UNICAMP - Brazil, Brazil

Atomic Force Microscopy (AFM) was used to study the morphology of crude venom from the South American rattlesnake Crotalus durissus terrificus. The effects of the crotalic venom on humans are systemic, leading to suffocation in fatal cases due to the neurotoxic, myotoxic and coagulative action of the components of the venom. We used adult snakes from the Pantanal region, Mato Grosso do Sul, Brazil, that remained without food for 30 days before the venom was extracted. The venom was collected manually by a specialist and dried at room temperature. Atomic Force Microscopy images, obtained using low vertical forces, allowed characterization of the surface morphology of the samples at sub-micron resolution. Coiled and porous structures are observed. Characterization of the venom by AFM is potentially of great importance because it may allow the comparison of its natural components. Critical discussion of the experimental results and characterization of the samples by AFM are given.

BI-TuP13 Role of Interfacial Water Structure on the Protein Resistant Properties of Oligo(ethylene glycol) Monolayers, ***B. Subramanian,*** *J. Yan, G.P. Lopez,* The University of New Mexico

Understanding the mechanism of protein adsorption at surfaces is an important issue in the field of biomedical materials, cellular adhesion and clinical diagnostics. Self-assembled monolayers (SAMs) of oligo(ethylene glycol)-terminated alkanethiols on gold are known to be protein resistant and represent a good model system to study the interactions of proteins with organic surfaces. Although these SAMs are resistant to protein adsorption, the mechanism by which these monolayers prevent protein adsorption is not yet established. Recently, it was suggested that protein resistance of these monolayers is a consequence of the formation of a structured interfacial water layer, which prevents direct contact between the surface and the protein. It was further suggested that, this might be a common mechanism for other monolayers, which show resistant to protein adsorption. It has been observed that, interfacial water undergo sharp changes in its properties (e.g., density, surface viscosity) at 15, 30, 45, and 60°C. These changes are attributed to the change in the structure of interfacial water at that temperature. We examine whether the change in the interfacial water structure at these characteristic temperatures affect the protein resistant properties of these monolayers, by carrying out protein adsorption on mixed monolayers of hexa(ethylene oxide)-terminated alkanethiols and methyl terminated alkanethiol (@chi@ @sub EG6@ = 0.44) as a function of temperature. The results show that, there is a sharp change in the protein adsorption behavior at 30±1°C. Below this temperature, there is no protein adsorption and above this temperature there is approximately a monolayer of protein adsorbed on the SAM surfaces. These results strongly support the view that interfacial water structure plays an important role in the protein resistant properties of oligo(ethylene glycol) SAMs.

BI-TuP16 Oligo(Ethylene Glycol)-Terminated Self Assembled Monolayers: Protein Resistance and the Effect of Assembly Temperature, ***C. Boozer,*** *S. Chen, L. Li, S. Jiang,* University of Washington

The rational design of protein resistant surfaces is a critical step in the ongoing development of biomaterials and biosensors, yet we lack a fundamental understanding of how such surfaces work. Here, we report a systematic study of the behavior of oligo(ethylene glycol)-terminated self-assembled monolayers (SAMs) prepared at a range of temperatures. The monolayers were formed by self-assembly of (EG)6-terminated thiols, in a heated (or cooled) methanol solution, on both single crystal and polycrystalline gold films. The films were characterized using atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), and infrared adsorption (FTIR). Protein adsorption on the OEG-terminated SAMs was studied using a home-built surface plasmon resonance (SPR) sensor. It was found that the ability of the OEG-terminated SAMs to resist protein adsorption from a buffer solution correlates with the temperature at which they were prepared. Protein adsorption studies were performed with both bovine serum albumin and fibrinogen, and in both cases we found that

protein resistance of the films was greatly diminished by increasing assembly temperature. A possible mechanism will be presented.

BI-TuP17 Determination of Adsorption Thermodynamics for Lysine Residues on Functionalized SAMs Using Surface Plasmon Resonance, ***V.N. Vernekar***, *R.A. Latour,* Clemson University

Although protein adsorption is key to many bioengineering problems, it is still not well understood. New comprehensive approaches to this problem are needed. In this study we take a systematic approach to address protein-surface adsorption by studying submolecular interactions of peptide residues with model surfaces. We hypothesize that combining the intermolecular thermodynamic contributions for peptide residue-surface adsorption with intramolecular residue-residue interactions will provide an approach to accurately predict overall protein adsorption. Accordingly, the objective of this study was to develop experimental techniques to measure residue-surface adsorption using surface plasmon resonance spectroscopy (SPR). The model residue-surface system selected for this initial study was poly-L-lysine (PL) and OH & COOH terminated Au-alkanethiol self assembled monolayers (SAMs). Preliminary studies were conducted to develop surface preparation and cleaning protocols necessary to obtain a stable SPR signal during the adsorption process. Adsorption studies were then conducted to measure the difference in signal as a function of surface functionality and PL concentration. Results show that the amount of adsorbed PL increases with increasing solution concentration, with the COOH-SAM adsorbing more PL than the OH-SAM for each concentration. These studies provide experimental data that is needed for calculating thermodynamic parameters for adsorption (Gibbs free energy, enthalpy, entropy) for each of these model systems. These values will be compared to results predicted from computational chemistry studies by others in our group for these same residue/surface systems.

Biomaterials
Room 102 - Session BI+SS-WeM

Biological Interface & Surface Science
Moderator: C. Wöll, Ruhr-University Bochum, Germany

8:20am **BI+SS-WeM1 Hybridization Reactions between Surface Attached Oligonucleotides and Complements from Solution**, ***W. Knoll***, *D. Kambhampati, T. Neumann, M. Chen,* Max-Planck-Institut für Polymerforschung, Germany **INVITED**

The quantitative evaluation of hybridization reactions between surface-attached 15mer oligonucleotides and their complements from solution will be described. Reaction kinetics, as well as equilibrium binding studies are conducted in order to reveal the association/dissociation mechanism. Different strategies to prepare the interfacial probe layers are tested and compared to each other: These are 1) direct coupling of the catcher oligonucleotides to a gold substrate by thiol groups, 2) a streptavidin monolayer-based coupling scheme via biotinylated probe oligos, 3) a similar approach but based on a commercial dextran-streptavidin structure, 4) 15mers attached to polymer brushes grown by a "grafting-from" approach, and finally 5) a layer prepared by electropolymerization of hydroxyphenol-derivatized oligonucleotides. In cases where surface plasmon spectroscopy was not sensitive enough for label-free detection of the hybridization we employed our recently developed surface-plasmon field-enhanced fluorescence spectroscopy. Different versions based on having either the catcher strand labeled, or the target, or both (e.g., for energy transfer studies) will be discussed. Parameters that are studied include the effect of temperature, ionic strength, mismatch (number of bases, position) length of complement, charge density (DNA versus PNA) etc.

9:00am **BI+SS-WeM3 Functionalization of Metal-Oxide-Based Biomaterials and Biosensor Surfaces by Molecular Self-Asssembly Processes**, ***M. Textor***, *S. Tosatti, M. Zwahlen, S. Finken, J.A Hubbell, G. Haehner,* Swiss Federal Institute of Technology (ETH), Switzerland

Modifications of metal oxide surfaces based on spontaneous adsorption of alkane phosphates and polycationic copolymers and subsequent film formation are shown to be potentially very useful for designing the chemical and biochemical properties of metallic implants and optical biosensors. Alkane phosphates were found to self-assemble on a number of transition metal surfaces such as titanium oxide, tantalum oxide and niobium oxide through direct coordination of the phosphate head group to high-valency metal cations. The chemical and structural properties of the adlayers were investigated using XPS, ToF-SIMS and NEXAFS. Introducing terminal functionalities other than methyl, e.g. hydroxy, amine or oligo(ethylene oxide) groups, allows one to tailor physico-chemical properties such as wettability, surface charge or the strength of protein-surface interactions. A second class of molecular assembly systems, PEG-grafted polycationic copolymers, spontaneously form monomolecular adlayers on negatively charged metal oxide surfaces, imposing high resistance towards biomolecule adsorption. Through further functionalization of the PEG-chains with biotin or peptide moieties, specific interactions of the treated oxide surface with streptavidin or with cell receptors can be induced while preserving the low degree of non-specific events. w-functionalized alkane phosphate SAMs, as well as peptide-modified PEG-grafted copolymers have been applied to both smooth and rough titanium surfaces to produce model surfaces for the study of fibroblast and osteoblast cell-surface interactions, with independent control of surface topography and chemistry. Furthermore, the two novel molecular assembly systems are shown to have a substantial potential for the reproducible and cost-effective modification of chips in optical-waveguide-based bioaffinity sensing of proteins and of DNA/RNA, including their application to microarray-type sensor surfaces.

9:20am **BI+SS-WeM4 Buildup Mechanism for Hyaluronic Acid/polysine Films onto a Solid Interface**, *C. Picart, Ph. Lavalle, F.J.G. Cuisinier,* INSERM U424, France; *P. Schaaf,* Institut Charles Sadron (CNRS) Strasbourg, France; ***J.C. Voegel***, INSERM U424, France

The formation of a new kind of biocompatible films based on Poly-L-Lysine and Hyaluronic Acid (PLL/HA) by alternate deposition of PLL and HA was investigated. It is shown that the driving force of the buildup process appears, as for "convential" polyelectrolyte multilayer systems, to be the alternate overcompensation of the surface charge after each PLL and HA deposition. The construction of (PLL/HA) films appears to take place over two buildup regimes. The first regime is characterized by the formation of isolated islands dispersed on the surface and which grow both by addition of new polyelectrolytes on their top and by mutual coalescence of the islands. The second regime sets in once a continuous film is formed at the after the 8th bilayer deposition in our working conditions. QCM measurements at different frequencies evidences a viscoelastic behavior of the films which have a shear viscosity of the order of 0.1 Pa.s. During this second regime the mass of the multilayer film increases in an exponential rather than in a linear way. This exponential growth is explained the diffusion of free PLL chains into the interior of the film when it is brought in contact with a PLL solution and by the diffusion out of the film of a fraction of these free chain followed by their interactions with HA chains at the outer limit of the multilayer when the film is further brought in contact with a HA solution. The diffusion of free PLL chains into the film is also found to be accompanied by an expulsion of water out of the film. This new kind of biocompatible film incorporating a natural polymer of the extracellular matrix and a widely used polypeptide makes it a potential candidate for cell-targeted action and for the coating of different types of surfaces, such as implants or capsules, in order to mimic a natural extracellular gel.

9:40am **BI+SS-WeM5 Orientational Effects and Surface Free Energies in the Amino Acids Adsorption Process onto Silicon-based Surfaces**, *G.L. Gambino,* ***C. Satriano***, *G. Marletta,* University of Catania, Italy

The present paper deals with the study of the adsorption process of Lysine (Lys) and Cysteine (Cys) from aqueous solutions as a function of the substrate structure and solutions pH. The substrate effect has been studied for three silicon-based substrates, i.e. silicon dioxide, poly(hydroxymethyl)siloxane (PHMS) and oxygen plasma-treated PHMS. The pH role on the adsorption process has been investigated by performing the incubations in the amino acid solutions both at their isoelectric pH and at the physiological pH, i.e. pH = 7.4. The in situ characterization of the substrates-amino acids molecules interaction was performed by means of the Quartz Crystal Microbalance with Dissipation (QCM-D) technique. On the other hand, ex-situ measurements were performed by means of Angular Resolved X-Ray Photoelectron Spectroscopy (ARXPS) and Contact Angle (CA) measurements. In particular, ARXPS, by varying the sampling depth from @tdA@10 nm to @tdA@2 nm, allowed to elaborate a coverage model, while the CA technique, by using three test liquids, allowed to calculate the surface free energies and their relative dispersive and acid-base contributions. QCM-D data shows that Lys and Cys strongly adsorb onto the plasma-treated PHMS while on the untreated PHMS surfaces the adsorption of both amino acids does not occur. ARXPS measurements indicate that the adsorbed molecules exhibit a preferential orientation respect to the plane of the surface, however no uniform coverage is obtained for any kind of substrate. Finally, the CA measurements indicate that the polar component of the surface free energy is directly related to both the amount and the orientation of the adsorbed amino acid molecules.

10:00am **BI+SS-WeM6 Surface-bound Liposomes for Biomedical Applications**, *P. Vermette,* CSIRO, Australia; *E. Gagnon,* Universite Laval, Canada; *L. Meagher,* CSIRO, Australia; *D. Dunstan,* Melbourne University, Australia; ***H.J. Griesser***, CSIRO, Australia; *C. Doillon,* Universite Laval, Canada

Injectable liposomes, in particular PEG-coated liposomes, are well known in the pharmaceutical industry for drug delivery. However, much of the drug never reaches the intended target site. We have developed methods for binding liposomes onto surfaces of biomedical devices for controlled local delivery of drugs adjacent to implanted biomedical devices. In this way we aim to reduce drug amounts and wastage, and control the local host response to the implant, a response which with most current biomaterials typically is dominated by fibrous tissue encapsulation. We have produced liposomes with encapsulated drugs and model substances, characterized them in terms of size and release performance, and bound them onto polymeric surfaces. PEGylated phospholipid liposomes were produced by extrusion through polycarbonate membranes of various pore sizes. The diameters of the liposomes were characterized by photon correlation spectroscopy. For binding liposomes, polymer surfaces were coated with streptavidin, which was used for affinity capture of biotinylated PEGylated liposomes. Streptavidin was covalently bound onto polymer surfaces via an amine plasma (glow discharge) polymer interlayer and a layer of polyacrylic acid, onto whose carboxylate groups the streptavidin was attached by carbodiimide chemistry. Detailed surface analyses were used to characterize and verify each step in the fabrication of the liposome coated surfaces. To test the in vivo efficacy of liposome coated biomaterials, an

angiogenesis promoting drug was encapsulated and the liposomes attached. Both in vitro and in vivo there occurred markedly enhanced angiogenesis. Another way of using the same concept may be to implant streptavidin coated biomedical implants and then inject drug-loaded liposomes. Experiments are currently underway to investigate whether circulating biotinylated liposomes can thus be enriched at an implant surface by affinity capture with surface-immobilized streptavidin.

10:20am **BI+SS-WeM7 Thiol-Reactive Surfaces for the Specific Immobilization of Biomolecules**, ***R.L. Cicero***, *D. Martin, S. McManus-Munoz, C.E.J. Dentinger, P. Kernen, P. Wagner,* Zyomyx Inc.

We present multi-component self-assembled monolayers on Au(111) surfaces that extend thiol-reactive groups and chemical moieties known to resist non-specific adsorption of proteins. Particular attention will be paid to the structural characterization of these surfaces using X-ray photoelectron spectroscopy, reflection absorption infrared spectroscopy and ellipsometry. We also investigate the effect surface density of thiol-reactive groups presented at the solid-liquid interface has with respect to homogeneity, efficiency and specificity of immobilizing thiol-containing biomolecules to these surfaces. Several methods for detecting and quantifying biomolecule immobilization are used including surface plasmon resonance spectrometry, radiometry and fluorimetry.

10:40am **BI+SS-WeM8 Control and Detection of Surface Immobilized DNA Interactions Using Electrostatically Assisted Surface Plasmon Resonance**, ***R.J. Heaton***, *A.W. Peterson, L.K. Wolf,* Boston University*; R.M. Georgiadis,* Boston University, usa

Using Surface Plasmon Resonance (SPR) spectroscopy, we demonstrate that the formation of an immobilized DNA film on gold can be controlled by non-Faradaic electrostatic charging. Furthermore, electrostatic charging can be used to enhance interactions between the immobilized probe film and target oligonucleotides in solution. By simply adjusting the potential across the surface thiol-modified single stranded oligonucleotide films can be formed with good reproducibility in coverage. The application of an attractive potential can speed up and maximize the hybridization of complementary and mismatched oligonucleotides to the probe film. We demonstrate the efficacy of this technology with application to both single area and array-mode SPR.

11:00am **BI+SS-WeM9 Non-Uniform Mixing in Fluid Surfaces**, ***J.S. Hovis***, *S.G. Boxer,* Stanford University

Cell membranes are two-dimensional heterogeneous fluid surfaces comprised of lipids, proteins, and carbohydrates. Understanding their organization at the molecular level is of critical importance for understanding cellular function. One of the key features, of the cell membrane is it's fluidity, which precludes long range order. However, due to the heterogeneous nature of the system it is possible that non-uniform mixing occurs, resulting in the local enhancement of certain membrane components. We will present results from our studies designed to probe for one type of domain, termed 'lipid rafts', using model membranes. In particular, we use supported lipid bilayers that are partitioned; the partitioning enables us to spatially contain the membrane components. By applying an electric field in the plane of the bilayer we can rearrange the membrane components in the partitioned regions. Sphingolipids and cholesterol, the major components of lipid rafts are electrically neutral and will not reorganize in response to a field; however, GM@sub 1@, a minor component, will as it has a net negative charge. Using epi-fluorescence microscopy we monitored the resultant electric field induced reorganization of the membrane components. Our results indicate that the reorganization of the GM@sub 1@ induces a reorganization of the sphingolipids and cholesterol. However, this reorganization does not appear to be concerted, suggesting that the rafts are not long-lived structures. That is, there is an increased propensity for certain components to be in close proximity to one another, but due to the fluid nature of the lipid bilayer, individual components are not in close proximity for long. This work will hopefully provide additional insight into understanding how non-uniform mixing occurs in these fluid surfaces and what the functional consequences are.

11:20am **BI+SS-WeM10 Vesicle to Supported Bilayer Transformation Kinetics; Influence from Vesicle Size, Temperature and Surface Support**, ***E. Reimhult***, *K. Dimitrievski, V.P. Zhdanov, F. Höök, B. Kasemo,* Chalmers University of Technology, Sweden

Supported phospholipid bilayers (SPB) on solid surfaces are biologically functional components of high current interest, e.g., for biosensors, tissue engineering, and basic science (Sackman, Science 271:43 (1996)). We investigate how the kinetics of vesicle to bilayer transformation on SiO@sub 2@ depend on vesicle size using small Extruded Unilamellar Vesicles (EUV; diameter~30-200 nm) and Small sonicated Unilamellar Vesicles (SUV; diameter~25 nm)) and temperature (T~5 to 30°C).). The experimental results are complemented by computer modeling and MC simulations. Our results reveal weak but significant vesicle size-dependent kinetics. The rate and completeness of the vesicle-to-bilayer transformation is strongly dependent on temperature and the vesicle-to-bilayer formation on SiO@sub 2@ can under certain circumstances be completely inhibited at low temperatures. In addition, the vesicle-surface interaction was investigated for various surfaces, including oxidized Au, Pt and Ti, which all demonstrate adsorption of vesicles in an intact state independent of vesicle size and temperature. The obtained results extend our previous studies at constant vesicle size and temperature (Keller et al, Phys Rev B 61: (3) 2291 (2000)) and constitute a platform that will significantly improve the possibility to control the process on µmm-nm fabricated surfaces, from which more complex functional supported bio-membranes are constructed.

11:40am **BI+SS-WeM11 Functional Tethered Lipid Membranes on Gold**, ***K. Bender***, Stanford University

A solid supported, biomimetic lipid bilayer was formed on a gold substrate by adsorbing lipid vesicles on a self-assembled monolayer (SAM) consisting of thiol-lipopeptides mixed with thiol-peptides. The membrane was bound to the surface by the thiol-lipopeptides, the thiol-peptides being used to change the surface concentration of thiol-lipopeptide and hence control membrane fluidity. The lipid bilayer was formed by fusion of L-@alpha@-Phoshatidylcholine (eggPC) liposomes onto the mixed thiol-lipopeptide / thiol-peptide SAM. The free lipids replenish the tethered lipid layer and also form the second layer to complete the bilayer. A functionalized lipid bilayer was formed by incorporating H@super +@-ATP-synthase (extracted and purified from spinach chloroplastes) with the eggPC. This enzyme is a membrane integral protein that can synthesize or hydrolyze adenosine triphosphate (ATP) from or to adenosine diphosphate (ADP) and in doing so pumps H@super +@ through the bilayer. Impedance spectroscopy measurements demonstrated that the enzyme had not lost its biological functionality and was still active. The formation of the lipid bilayer was detected by using surface plasmon resonance spectroscopy (SPS). Finally Annexin V, a pore forming protein, was immobilised on a lipid bilayer by using Ca@super 2+@-ions to bind (by chelation) the negatively charged parts of the protein to the negatively charged lipids (1,2-Dimyristoyl-sn-Glycero-3-Phoshatidylserin) in the bilayer. Annexin V as a non-integral protein and its function as a passive ion transporter through the lipid bilayer was used for comparison of the active ion transporter H@super +@-ATP-synthase. The same techniques as described above were used to observe the formation and activity of this system.

Organic Films and Devices
Room 131 - Session OF+NS+SS+BI-WeM
Self Assembled Monolayers/Ordered Films

Moderator: R. Maboudian, University of California, Berkeley

8:20am **OF+NS+SS+BI-WeM1 Preparation and Characterization of Nano-Scale Mixed Self-Assembled Monolayers**, *S. Chen, L. Li, C. Boozer,* ***S. Jiang***, University of Washington

Fabrication of nano-scale structures by mixed self-assembled monolayers (SAMs) has recently attracted much attention due to its scientific importance and potential applications to chemical and biological sensors, and biocompatible materials. However, it is still difficult to prepare nano-scale mixed SAMs since phase segregation occurs when two components are quite different. Recently, we proposed a new kinetically-trapped method to prepare nano-scale uniform mixed SAMs. In this work, we prepared various mixed SAMs, such as dodecanethiol(C12)/octanethiol(C8), tetradecanethiol (C14)/C8, 11-mercaptoundecanol(C11OH)/C8, and 11-mercaptoundecanoic acid(C10COOH)/C8 at a range of compositions using the kinetically-trapped method. Our results by low-current scanning tunneling microscopy (STM) revealed homogenous mixed SAMs with various terminal groups and a solution composition up to 25% of long chains formed at higher solution temperatures. Possible mechanism for forming uniform mixed SAMs will be discussed.

8:40am **OF+NS+SS+BI-WeM2 The Role of Linker Molecules in the Controlled Adsorption of Polystyrene (PS) Nano Particles**, ***M. Himmelhaus,*** Universität Heidelberg, Germany*; H. Takei,* Hitachi Central Research Laboratory, Japan

Controlled adsorption of PS nano particles onto specific regions of flat surfaces has found increasing interest as potential applications for surface-adsorbed PS particles, such as fabrication of quantum dots, optical switches, mesoscopic lasers, biosensors, as well as dosing of biomolecules, require an easily applicable adsorption scheme capable of addressing macroscopic areas. Among the various techniques those utilizing linker molecules to promote particle-particle as well as particle-surface interaction have only recently been applied as to date the role of the linker molecules in the various physical and chemical adsorption mechanisms is only poorly understood. We have studied the effect of several water-soluble linker molecules on the adsorption behavior and packing density of surfactant-free polystyrene latex spheres from suspension. By variation of several parameters, such as molarity of the linker molecules, pH of the suspension, as well as choosing differently functionalized PS particles we can distinguish several adsorption mechanisms from each other, reaching from purely physical ones to covalent bonding. This basic study is a first step to the fabrication of 2D crystalline monolayers of macroscopic lateral extension by means of chemically driven self-assembly.

9:00am **OF+NS+SS+BI-WeM3 Characterization of Biphenyl-substituted Alkanethiol Self-assembled Monolayers by High-resolution X-ray Photoelectron Spectroscopy**, *K. Heister, H.-T. Rong, M. Buck,* University Heidelberg, Germany*; L.S.O. Johansson,* University Karlstad, Sweden*;* ***M. Zharnikov****, M. Grunze,* University Heidelberg, Germany

Synchrotron-based high resolution X-ray photoelectron spectroscopy was applied to characterize self-assembled monolayers (SAM) of biphenyl-substituted alkanethiols CH@sub 3@(C@sub 6@H@sub 4@)@sub 2@(CH@sub 2@)@sub n@SH (BPn, n = 1-4) on Au and Ag substrates. Beyond previously identified odd-even changes in the packing density and the tilt angle of the biphenyl moieties, the high resolution spectra reveal a number of additional odd-even effects upon variation of the number of methylene groups in the aliphatic part in the BPn molecule. Their occurrence and mutual correlation suggests that a BPn SAM represents a strongly correlated, highly ordered molecular assembly. In particular, periodical changes of a shake-up feature in the C 1s region are observed, which are related to the differences in the arrangement of the aromatic matrix. The width and binding energy of the S 2p signals also exhibit odd-even changes. The width changes are associated with the occupation of either equivalent or non-equivalent adsorption sites on the polycrystalline (111) Au and Ag substrates. The comparison of the width values with those for conventional alkanethiols implies that the substrate bonding of alkanethiols on gold cannot be described by a single adsorption site. At the same time, the FWHM of the S 2p@sub 3/2,1/2@ peaks in the loosely packed BPn/Au (~0.50 eV) was found to be the smallest one among all thiol-derived SAMs investigated by HRXPS until present. Therefore, this value can be associated with the occupation of equivalent adsorption sites on the Au(111) surface. This work has been supported by the German BMBF (05 SF8VHA 1 and 05 SL8VHA 2), DAAD (313/S-PPP), and DFG (Bu820/11-2).

9:20am **OF+NS+SS+BI-WeM4 Separation via Self-assembly of Enantiomers of Chiral Aromatic Hydrocarbons Adsorbed on Metal Surfaces**, ***K.-H. Ernst,*** *Y. Kuster, R. Fasel,* EMPA Duebendorf, Switzerland

We studied the interaction of heptahelicene ([7]H), a helically shaped, polyaromatic phenanthrene derivative, with well-defined single-crystal metal surfaces under ultra high vacuum (UHV) conditions. The molecules, racemate as well as the pure enantiomers, were deposited via molecular beam technique and subsequently characterized with surface sensitive techniques like temperature programmed desorption (TPD), Auger electron spectroscopy (AES), X-ray photoelectron spectroscopy (XPS), low-energy electron diffraction (LEED), scanning tunneling microscopy (STM), time-of flight secondary mass spectrometry (ToF-SIMS), X-ray absorption spectroscopy (NEXAFS), and X-ray photoelectron diffraction (XPD). On Ni(111), the [7]H-molecule is adsorbed intactly at room temperature. TPD, however, shows only desorption of molecular [7]H from the multilayers. The first layer undergoes decomposition into carbon and hydrogen at 650 K. From Cu(111), molecular desorption is also observed at low coverages. The closed packed monolayers of [7]H on Ni(111) and Cu(111) show two-dimensional lattice structures. Adsorption of racemic [7]H leads to self-alignment into domaines on the surface, which are mirror images of each other. This is not observed after exposure to pure enantiomers and can be explained with a lateral separation of the enantiomers into homochiral domaines on the surface. For the pure M-enantiomer on the stepped Cu(332) surface, an azimuthal alignment of the molecular spirals is observed. Models for the monolayer structures and the mechanism of the separation will be discussed. Support by the Swiss National Science Foundation (NFP 36) is gratefully acknowledged.

9:40am **OF+NS+SS+BI-WeM5 Mesoscopic Correlation of Supramolecular Chirality in One-Dimensional Hydrogen-Bonded Assemblies**, ***J.V. Barth,*** Ecole Polytechnique Federale de Lausanne, Switzerland*; J. Weckesser,* Max-Planck-Institut fuer Festkoerperforschung, Germany*; A. De Vita,* Institut Romand de Recherche Numerique en Physique des Materiaux, Switzerland*; C. Cai,* University of Houston*; K. Kern,* Max-Planck-Institut fuer Festkoerperforschung, Germany

We studied enantioselective self-assembly in two dimensions employing the molecule 4-[trans-2-(pyrid-4-yl-vinyl)] benzoic acid. Scanning tunneling microscopy observations at noble metal surfaces reveal the formation of hydrogen-bonded supramolecular twin chains in two mirror-symmetric species, each containing only molecules of a given chirality. The twin chains are ordered in µmm-gratings, where a mesoscopic correlation of supramolecular chirality over the entire domain size without intimate molecular contact persists. This novel phenomenon reflects mesoscopic chiral segregation due to chiral recognition in the formation of the supramolecular assemblies. Theoretical modelling in conjunction with direct observations indicate that twin chains act as enantioselective templates for transient molecular attachment, which process mediates self-replication of supramolecular chirality and the enantiopure gratings' evolution.

10:00am **OF+NS+SS+BI-WeM6 Controlling Molecular Orientation in Solid Films Via Self-organization in the Liquid-crystalline Phase**, *I.K. Iverson, S.-W. Tam-Chang,* ***S.M. Casey,*** University of Nevada, Reno*; B.A. Pindzola,* University of California, Berkeley

We report the control of molecular orientation in solid films through self-organization and induced-orientation processes. We synthesized water-soluble cationic 3,4,9,10-perylene diimide (1) and studied its self-organization in aqueous solution. By UV-vis spectroscopy, H-aggregates of 1 are observed forming in solutions with concentrations as low as 10@super -7@ M. At concentrations above approximately 0.1 M (7% w/w) these solutions are observed with polarized microscopy to form a chromonic N phase (a nematic lyotropic liquid crystalline phase) at room temperature. Upon induced alignment (by shearing) of the chromonic N phase on a glass substrate and removal of solvent, anisotropic solid films of the dichroic dye are produced. These films have dichroic ratio values that routinely exceed 25 and in some cases 30, making them excellent sheet polarizers over the blue and green region. Using a combination of polarized UV-vis and FT-IR spectroscopies, the orientation of the average molecular plane in these films is determined to be perpendicular to both the shearing direction and the substrate plane. X-ray diffraction studies indicate that the molecules in the solid film possess a high degree of order.

10:40am **OF+NS+SS+BI-WeM8 Effect of Lipid Vesicle Fusion on the Ordering and Redox Activity of 11-(ferrocenyl carbonyloxy) Undecanethiols Self-assembled Monolayers**, *A.T.A. Jenkins,* University of Bath, U.K., Great Britain*;* ***J.F. Le Meur,*** University of Bath, U.K.

Self-assembled Monolayers (SAMs) of 11-(ferrocenyl carbonyloxy) undecanethiol were made following a procedure given by Chidsey et @footnote 1@ The formation of the 11-(ferrocenyl carbonyloxy) undecanethiol SAM on gold was followed in-situ by Surface Plasmon Resonance (SPR)and showed a film of thickness 13 Å was formed. Impedance measurements indicated a high level of film coverage. Cyclic voltammetry was subsequently used to electrochemically characterise the SAM, and check its stabilty with respect to immersion in electrolyte. Egg-Phosphatidylcholine lipid vesicles were created by extrusion through a 50 nm membrane and were adsorbed on the SAM. SPR was used to follow the lipid adsorption on the SAM. Cyclic voltammetry measurements on the SAM-lipid system showed a large and reproducible increase in the peak anodic and cathodic currents after lipid adsorption, although the total quantity of charge transfered stayed the same. This is likely to be due to an increase in order of the ferrocene units in the SAM, allowing for a faster transfer of electrons on the lipid covered SAM than the SAM alone. The above experiments were repeated with binary mixtures of SAMs containing both 11-(ferrocenyl carbonyloxy) undecanethiol and mercaptoundecanol moities. It was found that the increase in anodic and cathodic current maximums measuresed by cylic voltammetery was disproportionately lower than the single component SAM (relative to the coverage). From this we propose a model for how the SAM structure changes upon lipid

adsorption. @FootnoteText@ @footnote 1@ Chidsey, C.E.D.; Bertozzi, C.R.; Putvinski, T.M.; Mujsce, A.M. Journal American Chemical Society, 1990, 112, 4301-4306.

11:00am **OF+NS+SS+BI-WeM9 Temperature-dependent Morphology of Crystalline p-sexiphenyl Thin Films on KCl(001)**, ***E.J. Kintzel, Jr.,*** Florida State University*; D.-M. Smilgies,* Cornell University*; J.G. Skofronick, S.A. Safron,* Florida State University

Investigations of the morphology of ultrathin films of p-sexiphenyl (p-6P) vapor deposited onto KCl(001) have been carried out using the complementary techniques of X-Ray Diffraction (XRD) and Atomic Force Microscopy (AFM). XRD studies have shown that the molecular orientation of the p-6P is dependent on the substrate temperature during deposition. For films prepared at low temperatures, the p-6P molecules take a lying orientation, with the long axis of the molecule aligned parallel to the substrate. As the substrate temperature was increased during deposition, XRD results indicate two coexisting molecular orientations, corresponding to lying and standing p-6P molecules. AFM images provide independent confirming evidence of the influence of substrate temperature on molecular orientation, consistent with the XRD results.

11:40am **OF+NS+SS+BI-WeM11 Characterization of Photoisomerization Reaction of Azobenzene-contanining SAMs: Reaction Kinetics and Thermal Stability**, ***K. Tamada,*** National Institute of Advanced Industrial Science and Technology (AIST), and Frontier Research System, RIKEN, Japan*; H. Akiyama, T. Wei,* AIST, Japan

We studied the change of photoreactivity of azobenzene disulfide SAMs under thermal stress. Azobenzene-containing unsymmetrical disulfide (C6AzSSC12) SAM was annealed at each temperature (70, 85, 100, 120, 140°C) for 1 hr, then the photoreaction was monitored with surface plasmon resonance spectroscopy (SPR) in hexane. The photoreaction was stable under 100°C when no decomposition of adsorbed molecules was detected, while it was suddenly reduced at over 100°C when the decomposition was taking place. After annealing at 140°C, the reactivity of the unsymmetrical disulfide SAM reached to the level of the corresponding azobenzenethiol SAMs (single component), suggesting the phase segregation of the adsorbed molecules by annealing. We designed new azobenzene thiol/disulfides (C6Az(Me)thiol, C6Az(Me)SSC12) to achieve more thermally stable photoresponse. In these molecules, CH@sub 3@-group is introduced to the azobenzene ring directly to avoid dye aggregation sterically. The C6Az(Me)thiol SAM exhibited much higher photoreactivity than conventional azobenzenethiol, which suggests that dye functions are less aggregated even in the single component SAMs. The C6Az(Me)SSC12 exhibited much higher photoresponse compared with C6AzSSC12 after annealing, since these azo dyes can react even in phase segregated domains. We also discuss photoisomerization reaction kinetics (cis to trans, trans to cis) in correlation with dye alignment.

Biomaterials
Room 102 - Session BI+AS-WeA

Surface Characterization
Moderator: D.G. Castner, University of Washington

2:00pm **BI+AS-WeA1 Sum Frequency Generation (SFG) - Vibrational Spectroscopy and Atomic Force Microscopy (AFM) Studies of Biomaterial Liquid and Gas Interfaces. Surface Structures, Compositions and Bonding**, ***G.A. Somorjai,*** University of California, Berkeley **INVITED**

The surface monolayers of polyethylene and polypropylene and its blends, polyurethanes with different hydrophilic and hydrophobic endgroups and their blends and pHEMA have been studied by a combination of SFG and AFM. SFG reveals the different surface structures of polyolefins as a function of molecular weight. Changes of chain orientation occur at the glass transition temperature of polypropylene (-10 ËšC). When polyethylene is stretched, the surface becomes rough as the spherulites align in the stretch direction thereby weakening the polymer normal to the stretching direction. pHEMA with polymer groups that are crosslinked and polymer chains that are not exhibit variations of friction coefficients as measured by AFM. The low friction areas can be associated with polymer chains that are not crosslinked that can also be removed by methanol solution. These studies can also be carried out while the surface is under water. AFM reveals phase separation in blends due to the difference in friction coefficients of the two polymer components.

2:40pm **BI+AS-WeA3 Quantitative Analysis of Multicomponent Adsorbed Protein Films by Static Time of Flight Secondary Ion Mass Spectrometry**, ***M.S. Wagner,*** *M. Shen, T.A. Horbett, D.G. Castner,* University of Washington

Quantitative analysis of multicomponent adsorbed protein films is an integral part in the investigation of biofouling in many marine, food processing, and biomaterial applications. We have previously shown that Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) and multivariate analysis (MVA) are ideal techniques for the analysis of single component adsorbed protein films.@footnote 1@ MVA is also essential for quantitative compositional analysis of multicomponent adsorbed protein films in one parallel ToF-SIMS experiment. Partial Least Squares Regression (PLSR), a multivariate calibration technique, can quantitatively determine the composition of binary protein mixtures adsorbed onto mica using only the single component spectra for calibration. Current research focuses on binary protein mixtures on fluorocarbon and nitrogen-containing plasma polymer surfaces, both providing unique challenges for quantitative analysis by ToF-SIMS. Ternary and quaternary adsorbed protein films adsorbed onto mica were also investigated to determine the limit of complexity for quantitative compositional analysis. Finally, Principal Component Analysis (PCA) and a set of standard spectra were used to obtain semi-quantitative compositional analysis of a time series protein adsorption from dilute plasma solutions. Factor-deficient (fibrinogen and kininogen) plasmas were studied to corroborate this data. Multivariate analysis and ToF-SIMS are useful tools for quantitative compositional analysis of multicomponent adsorbed protein films. @FootnoteText@ @footnote 1@ M. S. Wagner and D. G. Castner, Langmuir in press (2001).

3:00pm **BI+AS-WeA4 Limits of Detection and Identification for Adsorbed Protein Films using XPS, ToF SIMS and Multivariate Analysis**, ***S.L. McArthur,*** *M.S. Wagner, M. Shen, T.A. Horbett, D.G. Castner,* University of Washington

In the ongoing development of biomaterial surfaces capable of resisting protein adsorption, surface analytical means of accurately detecting and subsequently identifying sub monolayer amounts of protein are becoming critical. The surface and chemical sensitivity of ultrahigh vacuum techniques such as XPS and ToF-SIMS have been utilized for some time in the detection of adsorbed protein films. More recently, multivariate analysis techniques have enabled the identification of the adsorbed species from both single and binary solutions via ToF-SIMS. In the instances where protein adsorption is low factors such as surface chemistry, surface coverage and roughness can be expected to complicate data interpretation, strongly influencing the both the detection sensitivity and accuracy of the protein identification. In this study a number of different surfaces were incubated in protein solutions of varying concentrations from 0.1ng/ml to 100µg/ml. Protein adsorption was quantified using radiolabelling and each surface analyzed using both XPS and ToF-SIMS. The results of the study illustrated the significant role of surface chemistry on the detection limits for adsorbed proteins. Not surprisingly, the presence of nitrogen in the substrate hindered the detection of protein by XPS, although detection limits remained high (<10ng/cm@super2@) for ToF-SIMS. The most interesting finding was the poor detection limits on PTFE surfaces, where both XPS and ToF-SIMS were unable to detect proteins below 100ng/cm@super2@. The detection limit of ToF-SIMS for protein adsorbed onto mica was 0.1 attomole of protein, rivaling the sensitivity of liquid and gas phase MS. Multivariate classification methods were also implemented to identify the adsorbed protein at submonolayer surface coverages. These results were also highly dependent on the substrate chemistry and morphology. Both XPS and ToF-SIMS are useful tools for the characterization of low levels of adsorbed protein.

3:40pm **BI+AS-WeA6 High z-resolution Microscopy of Biological Interfaces**, ***C.M. Ajo-Franklin,*** *L.C. Kam, S.G. Boxer,* Stanford University

Total internal reflection fluorescence microscopy (TIRFM) is widely used to study the structure and dynamics of biological interfaces by confining the excitation of a complex fluorescent sample very close to the material on which it is supported. By working with high refractive index solid supports, it is possible to even further confine the evanescent field, and by varying the angle of incidence, to profile fluorescent objects with high z-resolution. High refractive index materials, such as lithium niobate, sapphire, and zinc sulfide, exhibit different surface chemistries and each presents a unique challenge for defining biomolecular assemblies at the surface, a prerequisite for high resolution TIR techniques. In contrast, many well-developed strategies exist for modifying and tethering biomolecules to SiO@sub 2@ surfaces. Furthermore, SiO@sub 2@ surfaces are one of the few that can be used as substrates for supported lipid bilayers, a useful model system for studying biological membranes and interactions between membrane components and cells. We report the fabrication of hybrid surfaces consisting of nm layers of SiO@sub 2@ on lithium niobate (LiNbO@sub 3@, n = 2.3). Supported lipid bilayer membranes can be assembled and patterned on these hybrid surfaces as on conventional glass. By varying the angle of incidence of the excitation light, we show resolution of structures near a dielectric interface displaced by only tens of nanometers. These results demonstrate that it should be possible to profile the vertical location of fluorophores with nm resolution in real time, opening the possibility of many experiments at the interface between supported membranes and living cells.

4:00pm **BI+AS-WeA7 Chiral Recognition Observed at the Molecular Level by UHV-STM: Cysteine on Au(110)-(1x2)**, *A. Kühnle,* ***T.R. Linderoth,*** *B. Hammer, F. Besenbacher,* University of Aarhus, Denmark

Chirality is a frequently encountered property of organic molecules, leading to the existence of two mirror-image enantiomers. Interestingly, Nature is often homo-chiral in the sense that only one enantiomer participates in biological processes. Molecular recognition with chiral specificity is thus crucial within many fields of chemistry, biology and medicine. It is also essential for strategies to resolve racemic mixtures into enantiopure phases. Here we report on the adsorption of the chiral amino acid cysteine [HS-CH@sub 2@-CH(NH@sub 2@)-COOH] on the Au(110)-(1x2) surface under Ultra-High Vacuum conditions. Using Scanning Tunneling Microscopy (STM) we have discovered that the cysteine molecules can form isolated molecular pairs that break the mirror symmetry of the gold surface. This provides a model system for a molecular level study of chiral recognition: Deposition of the pure L and D- enantiomers, respectively, leads to identical, but mirror-reflected, cysteine pairs clearly distinguishable by STM. Most interestingly, deposition of the racemic D-L mixture only leads to the known homochiral pairs, showing that heterochiral D-L molecular interaction is avoided. To explore the origins of this novel chiral recognition mechanism, we have performed ab-initio DFT calculations. We find that the cysteine molecules are anchored to the surface via S-Au bonds and interact mutually through hydrogen bonds between the carboxylic groups. Importantly, a favorable interaction between the surface and the amino group is only geometrically feasible for homochiral pairs. The mechanism behind the observed chiral recognition is thus reminiscent of the generic so-called three-point contact model for chiral ligand-receptor interaction.

4:20pm **BI+AS-WeA8 Interactions between Calix-6-arene Sulfonates and Poly (Allylamine Hydrochloride) : A Stoichiometric Complexation Able to Release Proteins Bound to the Polyelectrolyte**, ***V. Ball,*** *G. Esposito, A.W. Coleman, P. Schaaf,* CNRS, France*; J.C. Voegel,* Unite INSERM 424, France

In the framework of our research, aimed to understand the fundamental mechanisms of interactions between polyelectrolytes and proteins or between polyelectrolytes and ions either in the adsorbed state on surfaces or in solution, we describe here the interaction between calix-6-arene

sulfonate (C6S) and poly (allylamin hydrochloride), PAH. At pH = 7.4, the C6S molecules carry 6 negative charges owing to their sulfonate groups whereas the polyelectrolyte is positively charged. Electrophoretic mobility experiments show that neutral particles are formed when the amount of negative charges coming from C6S matches the amount of positive charge from PAH (two chain lengths have been investigated). These particles display sizes in the µm range and are subjected to sedimentation after long term equilibration. Hence, coacervation occurs and this process is irreversible, since addition of either an excess of PAH or C6S does not change the solution turbidity and the particle size distribution. At smaller C6S/PAH stoichiometries, where C6S is totally removed from the unbound state in solution, the particles are positively charged and smaller in size (hydrodynamic radius between 50 and 150 nm). Therefore, the neutral particles obtained at the charge equivalence may result from aggregation of smaller C6S-PAH aggregates. Moreover, H-NMR experiments show that the C6S molecules are tightly bound to the polymer. We show then that one can make use this strong interaction in competition experiments to release quantitatively bovine serum albumin (BSA) that has been bound to PAH molecules in a previous step. These BSA-PAH aggregates are spontaneously dissociated and replaced by C6S-PAH particles which sediment, the supernatant only containing proteins in their native secondary conformation.

5:00pm **BI+AS-WeA10 Carbon Based Coatings for Cardiovascular Stents**, ***E.A. Evans,*** University of Akron*; U. Hafeli,* Cleveland Clinic Foundation

Carbon based films including diamond like carbon have been deposited as a protective layer for coronary stenting applications. Taking advantage of carbon's stability, or resistance to chemical attack, its flexibility, and other properties, our current technical objective is to develop carbon based materials as coatings for radioactive rhenium stents. Radioactive rhenium stents are being investigated to limit smooth muscle cell growth following coronary surgery. The carbon based coating is being investigated to reduce the release of radioactive rhenium (free activity) into the blood following the stenting procedure. Plasma enhanced deposition was used to deposit the carbon based coating onto metallic substrates. Foils, wires, and coils were coated and tested for adhesion, cytotoxicity, and release of radioactive rhenium. A critical requirement for successful stenting devices is a uniform surface over the entire structure. Our initial results indicate a 50% decrease in the release of free activity relative to uncoated rhenium. Relationships between deposition parameters and coating performance will be presented.

Biomaterials
Room 103 - Session BI+NS-WeA
Nanobiology

2:00pm **BI+NS-WeA1 Light-controlled Molecular Shuttles Based on Motor Proteins**, ***H. Hess,*** *J. Clemmens, D. Qin, J. Dennis, J. Howard, V. Vogel,* University of Washington

Molecular shuttles, an active transport system to position nanoscale objects, are needed as parts of molecular assembly stations, self-healing materials, or nanoscale actuators. The key problems of such a transport system are finding the motors, guiding the motion, loading cargo, and controlling the speed on the nanoscale. Active transport by single molecules is ubiquitous in biology and the solutions found by nature can serve as inspiration for technology. We demonstrate that molecular shuttles resembling conveyor belts can be constructed utilizing kinesin motor proteins as engines, microtubules as belts, and ATP as fuel. Two different strategies for guiding the microtubules have been explored by us: Arranging the motor proteins in nanometer-wide tracks by selective adsorption or creating micrometer-wide guiding channels by soft-lithography. Selective loading of cargo is accomplished by tagging cargo with streptavidin, and linking it to biotinylated microtubules. User-controlled exposure of caged ATP to UV-light and addition of an ATP-consuming enzyme to the buffer solution can move the microtubules in discrete steps. This forms a tool-set for the assembly of a functional molecular shuttle.

2:20pm **BI+NS-WeA2 The Direct Measurement of Drug-enzyme Interactions by Atomic Force Microscopy**, *S.M. Rigby-Singleton,* ***S.J.B.T. Tendler,*** *S. Allen,* University of Nottingham, UK*; M.C. Davies,* University of Nottingham, UK, United Kingdom*; C.J. Roberts, P.M. Williams,* University of Nottingham, UK

AFM has been employed to directly probe the rupture forces upon the mechanical dissociation of the drug-enzyme complex formed between the anticancer compound methotrexate and the protein dihydrofolate reductase (DHFR). AFM probes were functionalized with methotrexate immobilized beads and rupture forces recorded between the probe and a DHFR monolayer attached via Lys residues. Three variables were studied, AFM retraction rates, the presence of the enzyme cofactor NADPH and the protonation of the key enzyme Asp26 residue. Rupture forces of 91 pN were recorded at a retract velocity of 1 micrometer per second, a ten fold decrease in velocity resulted in an observed decrease in rupture force. The influence of the enzyme cofactor was negligible suggesting little effect on the dissociation pathway, this is in marked contrast to literature fluorescent binding assays. Notably a decrease in rupture force of approximately 25pN was observed when the pH was decreased below the pKa of the key Asp26 residue which is situated deep within the methotrexate binding site. These studies indicate that the AFM will be a valuable tool in the drug discovery process.

2:40pm **BI+NS-WeA3 Single and Multiple Molecule Binding Forces Measured Using Modified Atomic Force Microscope Cantilevers**, ***R.G. Rudnitsky,*** *F. Drees, K.S.H. Wu, T.D. Perez, W.J. Nelson, T.W. Kenny,* Stanford University

Although the energies and forces controlling protein interactions are frequently inferred from traditional equilibrium and kinetic measurements, recent developments in chemical force microscopy allow for the direct quantification of the ranges and magnitudes of binding forces between individual protein pairs and between groups of proteins. We report here on the use of specially modified Atomic Force Microscope cantilevers to measure bond strength down the single-molecule level, with pico-Newton force resolution, using the cellular binding protein E-cadherin as our model system. Previous E-cadherin studies focused on the energetics of large systems of molecules, typically in-vivo, to demonstrate their role in cellular adhesion. Our novel AFM force spectroscopy method tracks the unbinding process of single and multiple E-cadherin molecules under force loads, to quantitatively differentiate specific from non-specific binding, and single and multiple binding events, in surface bound protein. The measurements isolate the extracellular domain of the molecule, thought to be essential for stable cell adhesion, and demonstrate the dependence of binding forces at a molecular level on Ca++ concentrations. The data correlates the relationship of homophilic E-cadherin adhesion to surface protein density in a way not previously demonstrated in cellular studies.

3:00pm **BI+NS-WeA4 Selective Molecular Assembly Patterning - A New Approach to Micro- and Nanochemical Patterning of Surfaces for Biological Applications**, ***R. Michel,*** Laboratory for Surface Science and Technology, Switzerland*; J.W. Lussi,* Laboratory for Biomedical Engineering, Swiss Federal Institute of Technology, Zurich, Switzerland*; I. Reviakine, M. Textor, N.D. Spencer,* Laboratory for Surface Science and Technology, Switzerland

A novel method for producing chemically patterned surfaces based on selective self-assembly of alkane phosphates on metal oxide surfaces is presented. Standard photolithography is used to create patterns of titanium oxide within a matrix of silicon oxide by successively depositing 40 nm of TiO@sub 2@, 10 nm of SiO@sub 2@ onto a silicon wafer, followed by photoresist application and anisotropic etching. Ordered SAMs of alkane phosphates form on the TiO@sub 2@, but not on the SiO@sub 2@ surfaces by self-assembly. Poly-L-lysine-g-poly(ethylene glycol) (PLL-g-PEG) is used to render the exposed SiO@sub 2@ protein-resistant. X-ray photoelectron spectroscopy and imaging time-of-flight secondary ion mass spectrometry were used to characterize the surfaces. Protein adsorption studies conclusively established that the resulting surfaces presented protein adhesive (the TiO@sub 2@/alkane phosphate SAM region) and non-adhesive (the PLL-g-PEG-coated SiO@sub 2@) areas. This novel Selective Molecular Assembly Patterning (SMAP) technique was used to grow fibroblasts in the presence of serum on 5*5 µm TiO@sub 2@ spots. Cytoskeletal organization in the fibroblasts was induced above the 5*5 µm TiO@sub 2@ patches, while no interaction with the PLL-g-PEG background was evident. The SMAP technique is considered to be highly suitable for reproducible and cost-effective fabrication of biologically-relevant patterns over large areas, by combining state-of-the-art photolithography and

simple self-assembly dip-and-wash processes. Its applicability to sub-micrometer patterns is currently being evaluated.

3:20pm **BI+NS-WeA5 Studies of 20 nm Gold Particle Systems for Biosensing Applications & Optical Properties of Gold Nanostructures**, *L. Olofsson,* ***F. Höök****, P. Delsing, D.S. Sutherland, J. Gold, B. Kasemo,* Chalmers University of Technology, Sweden

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3:40pm **BI+NS-WeA6 Nanofabricated Lipid Bilayers Patterned on Metal Electrodes**, ***R.N. Orth****, I. Hafez, J. Kameoka, M. Lindau, H.G. Craighead,* Cornell University

Lipid molecules were immobilized on the surface of photolithographically patterned chromium and titanium. Large unilamellar lipid vesicles were found to bind on the native oxide surface of patterned support metals. Metal evaporation and resist liftoff techniques were used to pattern metal on a hydrophobic polymer surface. Lipids bound on solid substrates provide a biological interface for impedance measuring electrodes to detect bound cells or biomaterial. This patterning technique provides means to specifically bind lipids and conjugated biomaterials (polyethelene glycol (PEG), biotin, fluorescence dyes, and DNA oligimers) to the electrode surface. This technique may be applied to patterning biomaterial on metal inside thermally bonded microfluidic channels, to form titanium coated biomedical implants, and to create robust lipid-conjugated electrodes for biosensor applications.

4:00pm **BI+NS-WeA7 The Micropatterning of Mixed SAM Surfaces Using Inkjet Printing Technology: A Comparison Study to Microcontact Printing**, ***L.F. Pardo****, T. Boland,* Clemson University

Micropatterning is a powerful method for controlling surface properties, with a myriad of applications ranging from cell biology to electronics. Self-assembled monolayers (SAMs) of alkanethiolates on gold, the structures most widely used for preparing organic films with specific surface properties, are usually patterned by partitioning the surface into regions formed from different thiols. In microcontact printing for example, patterned self-assembled monolayers (SAMs) are printed onto a surface using a polydimethilsiloxane (PDMS), made using a microfabricated mold. Although this technique is suitable, the distortion of patterns, pattern limitation to binary mixtures and expensive mold design are limiting the efficient use of stamps. In this study, a new method utilizing inkjet printing technology for patterning mixed thiols is introduced. Methyl and carboxyl-terminated hexadecanethiols were patterned onto clean gold surfaces using a modified inkjet printer. The topography of the micropatterned samples was visualized and measured by atomic force microscopy. The chemico-physical properties investigated by Fourier Transform infrared spectroscopy and dynamic contact angle measurements suggest that inkjet printer yielded high throughput patterning on surfaces. This new inkjet printing technique provides a quick and inexpensive method for micropatterning alkanethiols of surfaces.

4:20pm **BI+NS-WeA8 Fabrication of High Aspect Ratio Vertically Aligned Carbon Nanofiber-based Electrochemical Probes for the Probing of Intact Whole Cells**, ***T. McKnight****,* Oak Ridge National Laboratory*; M.A. Guillorn,* Oak Ridge National Laboratory & University of Tennessee*; A.V. Melechko, D.W. Austin,* University of Tennessee*; V.I. Merkulov, M. Doktycz,* Oak Ridge National Laboratory*; D.H. Lowndes, M.L. Simpson,* Oak Ridge National Laboratory & University of Tennessee

Molecular biology and genomics are providing great insight into gene sequence, regulation and function. At the same time, imaging technology is elucidating cellular structure. Unfortunately, we have limited ability to monitor processes within and around living cells in real time and with high spatial resolution. This limitation is largely technological - our current research instruments are simply not on the same size scale as the functional components of cells. Here we present the fabrication and operation of high aspect ratio vertically aligned carbon nanofiber (VACNF)-based electrochemical probes for the probing of intact whole cells. Electron beam lithography was used to define the catalytic growth sites of the VACNFs. Following catalyst deposition, VACNF were grown using a novel plasma enhanced chemical vapor deposition (PECVD) process. Photolithography was performed to realize interconnect structures. These probes were passivated with a thin layer of SiO2, which was then removed from the tips of the VACNF, rendering them electrochemically active. We have demonstrated their functionality by selectively electrodepositing Au clusters onto the tips of the probes. We believe that these probes are ideally suited for characterizing intracellular phenomena in real time with an unprecedented degree of spatial resolution.

Biomaterials
Room 102 - Session BI-ThM

Protein Surface Interaction
Moderator: B.D. Ratner, University of Washington

8:20am **BI-ThM1 Physicochemical Properties of Polysaccharide Coatings as Determinants of Protein Adsorption**, *P.G. Hartley,* CSIRO Molecular Science, Australia; ***S.L. McArthur,*** University of Washington; *K.M. McLean,* CSIRO Molecular Science, Australia; *S. Oiseth,* Chalmers University of Technology, Sweden; *G. Johnson, H.J. Griesser,* CSIRO Molecular Science, Australia **INVITED**

The use of protein resistant coatings has long been seen as a means of controlling the biological response to implanted materials. Whilst many such surfaces have been produced, the properties which give rise to their protein resistant character are often poorly elucidated. Polysaccharides have been frequently employed as surface modification agents in the biomaterials area by virtue of their ready availability and apparent protein repellancy. The ability to chemically derivatize polysaccharides is a further key feature which suits them to studies of the relationship between surface chemistry and protein adsorption. In our studies we have utilized this ability to produce a range of derivatized dextran coatings with variable physico-chemical properties. These properties have been characterized in detail using both aqueous and high vacuum surface analytical techniques. These results have then been correlated with the protein adsorption behaviour of the surfaces. The results highlight the interplay between surface charge and steric interactions in determining the protein selectivity and/or repellency of the polysaccharide surfaces. In addition, we have further correlated the surface properties with biological responses using in vitro cell adhesion and growth studies. These studies demonstrate that control over cellular responses may be achieved to a large extent by the manipulation of non-specific interactions between polysaccharide surfaces and extracellular matrix proteins.

9:00am **BI-ThM3 Effect of Adsorbate Alkyl Chain Length and Terminal Group Chemistry on the Adsorption of Fibronectin and Albumin on Self-assembled Monolayers**, ***C.M.-J. Fauroux,*** *C.C. Dupont-Gillain, R.W. Manning,* UMIST, U.K.; *G.J. Leggett,* UMIST, U.K., UK

Recent studies of the responses of mammalian cells to self-assembled monolayers (SAMs) have provided important insights into the relationship between surface chemical structure and cell attachment to artificial surfaces. Our hypothesis is that a mechanistic explanation of the correlations we have observed depends upon a detailed knowledge of the composition of the protein layer that coats the SAM prior to cell attachment and the conformations of the molecules of which it is composed. Of particular importance are fibronectin (fn), which interacts with membrane receptors (integrins) involved in cell attachment, and albumin (alb), the must abundant component of serum but thought to inhibit attachment. We have studied the adsorption of these proteins onto a range of SAMs to determine whether there is a correlation between the results of our earlier studies and the kinetics of adsorption of these proteins. The kinetics of adsorption of single proteins (human alb and human fn) have been studied for methyl, hydroxyl and carboxylic acid terminated SAMs with short and long alkyl chains. Two complementary techniques have been used. Using @super 3@H-radiolabelling, the mass of adsorbed molecules per unit area may be determined. Measurement of the amide band intensity in Fourier transform infra-red spectroscopy (FTIR) also provides a measure of the amount of adsorbed protein. Data obtained by the two methods have been found to be in close agreement. It has been found that more alb adsorbed to methyl terminated SAMs than to carboxylic acid terminated SAMs, while the smallest amounts of adsorbed protein were observed for the hydroxyl terminated surfaces.

9:20am **BI-ThM4 Deformation of Proteins Adsorbed on Glass Surfaces as Characterized by XAS**, ***H.E. Canavan,*** George Washington University; *J.J. Hickman,* Clemson University; *W.E. O'Grady,* U.S. Naval Research Laboratory; *D.E. Ramaker,* George Washington University

The interaction of proteins with artificial surfaces is of interest to many in the fields of medicine, biotechnology, and surface science. It is known that certain proteins experience considerable conformational deformation upon adsorption onto surfaces. In contrast, some proteins are described as colloidal or "hard," and experience little if any deformation upon adsorption. In the work presented here, the biomolecular interaction is characterized by X-Ray Absorption Spectroscopy (XAS). Sulfur K-edge XAS will be used to analyze the S-S, S-C and S-O bonds to monitor the extent to which the sulfur bond character is changed in both "hard" and "soft" proteins such as BSA, lysozyme, and cytochrome C upon their adsorption onto a glass surface. In addition, X-ray Photoelectron Spectroscopy (XPS) is used to characterize the glass surfaces both prior and subsequent to protein deposition.

10:00am **BI-ThM6 Reversible Adsorption/Desorption of Proteins from a Thermally Switching Polymer Monolayer**, ***D.L. Huber,*** *M.A. Samara, B.C. Bunker, R.A. Manginell, C.M. Matzke, G. Dulleck,* Sandia National Laboratories

The phase transitions of poly(N-isopropyl acrylamide) (poly NIPAM) hydrogels have been studied extensively for a number of years. We have investigated the thermal transitions of the linear polymer bound to silicon oxide surfaces. The poly NIPAM monolayers are grown from a self assembled monolayer of free radical initiators, and their properties towards protein adsorption are studied as a function of temperature using IR and UV-visible spectroscopies, as well as ellipsometry and fluorescence microscopy. At room temperature, the monolayers are swollen with water and are extremely resistant to protein adhesion, but at elevated temperatures (above 35C) the polymer collapses and expels a large portion of the water. The collapsed polymer monolayers are capable of quickly adsorbing a protein monolayer. The layer of adsorbed protein can be completely desorbed by cooling the polymer to below its transition temperature. A well prepared monolayer has been shown to be capable of repeated adsorption and desorption cycles with no degradation of the effect. Poly NIPAM monolayers have been grown onto a microchip based platform containing micron scale resistive heaters capable of precisely controlling the surface temperature, and the adsorption and desorption of fluorescently labelled proteins monitored using flurorescence microscopy. Possible applications of on chip structures, as well as the adsorption/desorption kinetics will be discussed.

10:40am **BI-ThM8 Polyelectrolyte Multilayers : A New Tool to Design Targeted Biofilms**, ***P. Schaaf,*** Institut Charles Sadron / Universite Louis Pasteur Strasbourg, France; *L. Szyk,* Unite INSERM U424 Strasbourg, France; *B. Tinland,* Institut Charles Sadron (CNRS) Strasbourg, France; *F. Cuisinier, P. Schwinte, J.C. Voegel,* Unite INSERM U424 Strasbourg, France

The alternate deposition of polycations and polyanions on a solid surface allows to build a polyelectrolyte multilayer film. This method whose driving force is the charge overcompensation at each adsorption step, offers a simple and elegand way to design new types of films with applications ranging from non linear optics to nanoreactors. The buildup procedure also offers the possibility to develop new bioactive films with multiple functionalities. One can, for example, easily embed proteins into these films. We will present results relative to this later aspect and in particular to the structure and the diffusion of proteins embedded in multilayers. It will be shown that proteins embedded in multilayers are not irreversibly fixed but can diffuse along the film. The diffusion coefficient depends upon the polyelectrolytes in contact with the protein. Such films seem also to preserve the secondary structure of the adsorbed and embedded proteins an even to enhance their thermal stability. Polyelectrolyte multilayers appear also to inhibit the formation of intermolecular beta-sheets frequently observed during the heating of protein solutions. Some new perspectives of these films for the coating of biomaterials will finally be presented.

11:00am **BI-ThM9 Design of Bioadhesive Polymers for Use at Mucosal Interfaces**, ***A. Hoffman,*** University of Washington **INVITED**

Mucosal surfaces of the body include "wet" surfaces such as the eye, nose, mouth, GI-tract, vagina and lungs. They represent a large surface area of the body and thus may be an attractive route for delivery of drugs. When a drug formulation is applied to those surfaces, it may resist being washed away due to a combination of its own viscosity plus its intermolecular interactions with the mucous polymer coating. Two typical bioadhesive polymers that have been most often applied for mucosal drug delivery are polyacrylic acid and chitosan. This talk will describe drug delivery formulations containing PAA or chitosan or their derivatives that may provide better control over drug release rate and duration.

11:40am **BI-ThM11 The Role of Protein-surface Interactions in Implanted Joints**, *M.R. Widmer, M. Heuberger, J. Voros,* ***N.D. Spencer,*** ETH-Zurich, Switzerland

Proteins appear to play an important role in the boundary lubrication of both natural and implanted hip and knee joints. However, the nature of the interaction of proteins in synovial fluid with the prosthetic tribosurface

appears to influence the effectiveness of boundary lubrication significantly. Protein adsorption (waveguide and fluorescence experiments) and tribological (pin-on-disk) studies have been carried out on a number of polymer and model surfaces in order to determine the tribological role and nature of such interactions.

Thin Films
Room 123 - Session TF+BI-ThM

Bioactive and Organic/Inorganic Thin Films

Moderator: L. Hultman, Linköping University, Sweden

8:20am **TF+BI-ThM1 Self-aligned Deposition and Patterning of Biologically-active Polymer Thin Films**, ***B.H. Augustine***, *S.M. Ramirez, O.D. Lees,* James Madison University

High-resolution patterning and microfabrication of polymeric and other soft materials is challenging since traditional photolithographic methods require organic solvents to remove photoresist. These solvents typically also dissolve or degrade biological and polymeric surfaces which one might pattern. We report selective dewetting using microcontact printing (μ-CP), micromolding in capillaries (MIMIC), and solvent assisted micromolding (SAMIM) techniques to pattern thin films of the biodegradable copolymer, poly-3-hydroxybutyrate-co-3-hydroxyvalerate [P(3HB-3HV)] onto glass, silicon, and Au coated silicon substrates. Film thicknesses range from 20 mn to over 700 nm, with minimum feature sizes as small as 3 μm. Dense 100 nm thick films with sub-10 μm features can be patterned in as few as two minutes for the entire processing resulting in potentially high throughput processing. Thin film microstructure can be dramatically changed by controlling deposition parameters such as solvent concentration, feature aspect ratio, and polarity of the solvent. While we report microfabrication techniqes for a specific biodegradable polymer system, we will also comment on extending these techniques to other polymer systems and the issues affecting the profound change in polymer microstructure using these three different patterning techniques.

9:00am **TF+BI-ThM3 Desorption and Processing of Bioactive Thin Films**, ***A. Chilkoti,*** Duke University **INVITED**

I will describe methods to micro- and nano-pattern proteins and other biological ligands onto self-assembled monolayers (SAMs) and polymers for application in multianalyte biosensors, patterned biomaterials, and protein chips. These methods include: (1) Light-activated micropatterning (LAMP), which exploits spatially precise, light-activated deprotection of affinity ligands on functionalized SAMs to achieve step-and-repeat patterning of multiple biomolecules. (2) Microstamping onto activated polymer surfaces (MAPS), which involves surface-selective functionalization of polymers, followed by microcontact printing of reactive biological ligands. (3) Thermodynamically addressable reversible patterning (TRAP) which uses patterned domains with different surface energies as a thermodynamic address to direct the attachment of proteins and other biomolecules from solution. TRAP functions by the selective adsorption of nanoclusters of an elastin fusion protein above its phase transition temperature specifically on patterned hydrophobic regions, but not on a hydrophilic background. Unlike other methods for protein patterning, TRAP is reversible, and modulating the solution environment (e.g., T, ionic strength), can erase protein patterns. A theme illustrated by this talk will be the interdisciplinary convergence of surface chemistry and spectroscopic characterization (XPS, TOF-SIMS, and evanescent optical techniques) with molecular biology.

9:40am **TF+BI-ThM5 Nano-scale Fabrication Using Organic Thin Films**, ***C.B. Gorman,*** North Carolina State University **INVITED**

We will show how a combination of lithographic methods on organic self-assembled monolayers (SAMs) can be used to form chemically well-defined, patterned surfaces. These surfaces can form the basis of nanometer-scale, molecular electronic devices. The talk will focus on (1)the engineering and the chemistry behind nanometer scale lithography on SAMs including an assessment of its strengths and limitations, (2) why the control of chemical functionality is so important for a true, nanometer-scale process and (3) demonstration of new, molecular electronic behaviors with potential applicability in devices.

10:20am **TF+BI-ThM7 Hot-Filament Chemical Vapor Deposition of Fluorocarbon-Organosilicon Copolymer Thin Films**, ***S.K. Murthy***, *K.K. Gleason,* Massachusetts Institute of Technology

Hot-filament chemical vapor deposition, a non-plasma technique, has been used to deposit copolymer thin films consisting of fluorocarbon (CF@sub 2@) groups and organosilicon groups (Si(CH@sub 3@)@sub 2@ - O) at rates of approximately 250 angstroms/min. The synthesis of such copolymers by solution chemistry techniques is difficult since one component (PTFE) is normally synthesized by free radical polymerization techniques and the other (PDMS) by ionic polymerization methods. The presence of covalent bonds between the fluorocarbon and organosilicon moieties in the thin films has been confirmed by Infrared, X-Ray Photoelectron (XPS) and solid-state @super 29@Si, @super 19@F, and @super 13@C Nuclear Magnetic Resonance (NMR) spectroscopy. These techniques also indicate retention of methyl groups from the siloxane precursor. The XPS data shows that all of the silicon present in the films is in the +2 oxidation state and that the ratio of silicon to CF@sub 2@ groups is approximately 1:0.86 based on atomic composition. Further, the NMR data suggest that the copolymer films are blocky in nature, consisting of networked chains having multiple fluorocarbon groups interspersed between siloxane groups. Atomic Force Microscopy of the films showed that the roughness of these copolymer films is in-between that of homopolymeric fluorocarbon and organosilicon films made by the same technique.

10:40am **TF+BI-ThM8 Polyatomic Ion Deposition of Gradient Thin Films: A New Method for Combinatorial Materials**, ***L. Hanley***, *M.B.J. Wijesundara, E.R. Fuoco,* University of Illinois at Chicago

Beams of gaseous ions are used for the growth and modification of interfaces in a wide variety of applications. For example, we have previously shown that mass-selected CF@sub3@@super+@, C@sub3@F@sub5@@super+@, and Si@sub2@O(CH@sub3@)@sub3@@super+@ ions can be employed for the growth and modification of organic thin films on polymer and metal surfaces.@footnote1@We demonstrate here that polyatomic ion beams can also be employed to create chemical gradient thin films by variation of the ion fluence across the substrate. We use mass-selected C@sub3@F@sub5@@super+@ ion deposition in vacuum to create a fluorocarbon gradient film on a polymethylmethacrylate substrate. X-ray photoelectron spectroscopy shows a continuous change in the surface chemistry from that of the native polymer to a fluorocarbon film. The contact angle varies from ~75° to ~95° across the gradient surface. We also examine the production of fluorocarbon films on polystyrene, silicon, and aluminum surfaces from C@sub3@F@sub5@@super+@ ions. Finally, we discuss the general feasibility of producing chemical gradients surfaces from polyatomic ion beams. @footnote1@M.B.J. Wijesundara, Y. Ji, B. Ni, S.B. Sinnott, L. Hanley, J. Appl. Phys. 88 (2000) 5004

11:00am **TF+BI-ThM9 Plasma Sputtering Deposition of Metals on PAMAM Dendrimer Monolayer**, *A. Rar, M. Curry, F. Xu, J.A. Barnard,* ***S.C. Street***, University of Alabama

A number of nanotechnology applications require development of thin, flat surface films with well-regulated mechanical and tribological properties. A promising approach for this is metal layer deposition on PAMAM dendrimer underlayers. Previously, we demonstrated improvement in mechanical and morphological properties for Au, Co, and Cr films deposited by evaporation onto dendrimer self-assembled monolayers. In this paper we will discuss formation of metallic layers on dendrimer by plasma sputtering deposition. We will show the influence of higher incoming kinetic energy of the metal atoms on dendrimer structure and chemical changes at the interface. The evolution of the dendrimer interlayer during metal deposition was analyzed with XRR, the surface morphology of deposited films with AFM, the chemical interaction between deposited metal and dendrimers with XPS and RAIRS. Thin Cr layers obtained by plasma sputtering interact with the dendrimer interlayer in essentially the same way as films deposited by evaporation. Significant differences were found for Cu/dendrimer layers prepared by plasma sputtering deposition compared to less energetic thermal evaporation. In the first case more than 1/3 of the nitrogen atoms in the dendrimer adlayer form nitride-like chemical states. Thermal evaporation shows less pronounced influence on the N1s XPS peak.

Biomaterials
Room 102 - Session BI-ThA

Cell-Surface Interaction
Moderator: G.J. Leggett, UMIST, UK

2:40pm **BI-ThA3 Developmental Studies of Electrical Activity of Artificially Constructed Neuronal Cell Networks**, ***C.D. James***, *A.J. Spence, H.G. Craighead, M.S. Isaacson,* Cornell University*; N. Dowell, W. Shain, J. Turner,* Wadsworth Center

The hippocampus has been implicated in a range of brain functions such as the internal representation of space and memory consolidation. Dissociated hippocampal pyramidal cell cultures have yielded vital information about single unit and small network electrophysiology, yet monitoring synaptogenesis and the development of electrical activity within cell networks has proved to be a difficult task. The construction of neuronal cell networks has been investigated by many researchers for this purpose, and our labs have utilized microcontact printing and microfabricated electrode arrays to construct and study cell networks. Selective spatial organization of proteins and molecules have been used to direct neuronal cell attachment and neurite outgrowth in vitro, while microelectrode arrays allow long-term, non-invasive studies on developing network populations. The combination of both technologies has allowed our labs to monitor field and action potentials of designed cell networks in order to investigate the relevance of such factors as cell morphology and neuron-substrate interaction on the development and stability of connected units. Multi- and single-unit extracellular potentials of 50 to 300 microvolts have been observed and recorded with five simultaneous channels to enable single unit discrimination. Whole cell recordings were also performed to provide guidance in isolating single units in our extracellular recordings, while immunochemical staining of networks for synaptic proteins such as synaptophysin and PSD-95 was used to identify putative synapses. We believe that such studies may be able to provide valuable information about the maturation of coordinated activity between cells, primarily in regards to the influence of neuron morphology on action potential invasion into the somato-dendritic regions of firing cells, as well as on the developmental segregation and distribution within cells of relevant molecules such as ion channels and synaptic proteins.

3:00pm **BI-ThA4 The Affects of Geometric Constraints on Neuronal Process Extension**, ***A.M.P. Turner***, *S.W.P. Turner, R. Terao, H.G. Craighead,* Cornell University*; N. Dowell, W. Shain,* NYS DOH Wadsworth Center*; G. Withers, G. Banker,* Oregon Health Sciences University

Our research has involved the study of how central nervous system (CNS) cells attach to and grow on surfaces topographically modified with micrometer-sized features. In particular, we have studied the growth of rat hippocampal neurons on surfaces patterned with pillars. Patterned silicon substrates were made using conventional semiconductor methods and polymer embossing techniques were used to make transparent substrates. It was observed that the geometric constraints to which a neuron is exposed have a significant impact on various aspects of neuronal process development, including the rate of neurite (dendritic and axonal) outgrowth, neurite morphology, dendritic branching, and specific protein production, transport and organization. Fluorescence, scanning electron, and phase-contrast time-lapse microscopies were used to analyze and quantify the growth of neurons on surfaces with 1 to 2 μm tall pillars of various widths, 500 nm to 2 μm, and inter-pillar spacings, 1.0 μm to 5 μm. We observed a 50 percent increase in the rate of neurite outgrowth on surfaces with pillars versus smooth surfaces. It was also observed that in arrays with spacings less than 2 μm, the majority of neurites grow along 90 and 45 degree paths from the soma whereas with spacings of 4 μm and greater, neurites revert back to morphologies observed on smooth surfaces. Dendritic branching was found to increase with a decrease in inter-pillar spacing and immunochemical staining demonstrated various correlations between protein organization and pillar locations. The goal of these studies is to learn more about the fundamental interactions between CNS cells and surface structure.

3:20pm **BI-ThA5 The Use of Surface Composition to Control Cell Phenotype Expression**, ***J.J. Hickman***, *P. Molnar, G. Jacob, M. Das, T. Tauber,* Clemson University

There is currently a large amount of interest in neuronal STEM cell manipulation to create stable phenotypes. The initial phase of CNS development is characterized by the proliferation of the precursor cells, followed by the generation of neurons and glia. The neurons are differentiated into different neurotransmitter phenotypes as well as glial cells. However, the factors that control the differentiation of the precursor cells into differentiated cell types are still mainly unknown. It is believed that the cell environment plays a key role in the specification of neuronal cells, even though a cell intrinsic developmental program is important in regulating cell lineage. We have shown it may be possible to manipulate the development of specific phenotypes through cell-surface interactions. In the present study, the expression of neuronal cell phenotype was examined in a defined in vitro system in which embryonic rat cortical cells were grown on silica substrates modified with artificial surfaces composed of silane self-assembled monolayers (SAMs) in serum-free medium. Experiments were conducted utilizing various neurotrophic factors and various substrates to examine cortical neuron phenotype expression. Cultures were immunostained with a panel of antibodies to detect specific differentiation markers. On poly-D-lysine and DETA, glutamatergic cells represented 30-40% of total cells and GABAergic cells represented about 50-60% of total cells, which is consistent with immunocytochemical findings in vivo. On 13F the ratio of glutamatergic to GABAergic was greater. We will present these results as well as an explaination for the observed effects.

3:40pm **BI-ThA6 Stretching and Fibroblast Growth on GRGDSP-Peptide Modified Silicone Membranes**, *L. Hanley,* ***S.S. Lateef***, *S. Boateng, T.J. Hartman, C. Crot, B. Russell,* University of Illinois at Chicago

Diseased, hypertrophic human heart muscle cells (cardiac myocytes) are found to increase in length and volume due to excessive mechanical load. We are developing an entirely new cell culture in silicone elastomer that will mimic the in-vivo cell phenotype to address such questions in cardiac mechanobiology. We chemically modify silicone membranes to improve their ability to culture cardiac myocytes under dynamic stretching, thereby allowing study of mechanical effects. It is well known from studies of cellular attachment that several intrinsic proteins found on the cell surface will recognize and attach to the GRGDSP peptide sequence. We plasma oxidize the surface of the silicone membrane; functionalize it with amine via reaction with 3-aminopropyltriethoxysilane; attach a sulfo-maleimide cross-linker; then attach a 15-residue peptide, acetyl-CGGEGYGEGRGDSPG-amide, to the cross-linker through its terminal thiol group. The membranes are characterized by x-ray photoelectron spectroscopy, spectochemical analysis, and radiolabelling. Stretching studies with radiolabelled cysteine (in place of peptide) show that the modified layer survives on the surface for 48 hours of stretching in cell culture media. The GRGDSP peptide bound silicone shows enhanced binding of rat fibroblasts when compared with amine-functionalized and unmodified silicone surfaces.

4:00pm **BI-ThA7 Surface Characterization and HCAEC Adhesion Studies of IPN Modified 316 L Stainless Steel**, ***G.M. Harbers***, Northwestern University*; T.A. Barber, M.E. Yanez, H.B. Larman,* University of California, Berkeley*; K.E. Healy,* University of California, Berkeley, U.S.A

Interactions between synthetic biomaterials and components of the cardiovascular system still remain poorly understood. In particular, the process of restenosis following intravascular stent deployment remains a significant problem. Coatings that minimize protein adsorption and monocyte adhesion and proliferation may reduce late-term in-stent restenosis and prevent secondary interventions. In this work, a previously developed non-fouling P(AAm-co-EG/AA) interpenetrating polymer network (IPN) was applied to clinically relevant cardiovascular stent material (316L SS). The transfer of the technology from previous substrates (quartz, TiO@sub 2@/Ti, polystyrene) to SS was confirmed using water contact angle goniometry, XPS, and cell-material interactions. Water contact angle data was similar to what was previously reported for quartz substrates and XPS confirmed the addition of each subsequent layer. To test the ability of the modified material to resist cell adhesion, substrates were seeded with primary human coronary artery endothelial cells (HCAECs). Following a 24h incubation, cells were labeled and examined using fluorescent microscopy. HCAECs adhered to both the unmodified SS and the positive control (TCPS) but not to the IPN modified material (TCPS>SS>>IPN~PEG(NH@sub 2@)@sub 2@; 8875±2128, 6972±721, 124±22, and 99±29 cells/cm@super 2@ on respective surfaces). Cells on unmodified SS coupons had a similar morphology to those seeded onto TCPS. However, the few viable cells that attached to the IPN and PEG(NH@sub 2@)@sub 2@ remained spherical and non-spread. It has been proposed that endothelialization of the stent surface can improve stent performance by creating a native tissue layer. Therefore, since the IPN/316L SS system is amenable to peptide modification, the identification

of endothelial cell specific peptides to promote preferential endothelial cell adhesion, migration, and proliferation is under investigation.

4:20pm **BI-ThA8 Improved Functionalization for Chemically Patterned Polystyrene Surfaces**, *A.A. Meyer-Plath,* ***K. Schröder,*** *A. Ohl,* Institute of Non-thermal Plasma Physics, Germany

Tight contact between living cells and polymeric materials is a key characteristic of implant materials, medical, pharmaceutical diagnostic devices, and in vitro cell culturing. To improve cell adhesion and growth, surface modification is required for almost all types of polymer. Plasma-chemically introduced functional groups are widely used for this purpose. Type and density of surface functionalities control adsorption behaviour of cell-signaling molecules. Also, selective immobilization of biologically active molecules (e.g. attachment factors) is possible. This way, the polymer surface provokes cell responses. Chemical patterns for different cellular responses are the basis for some advanced applications of biomaterials. They may directly induce selective cell adhesion. Plasma functionalization is the basis for pattern generation. Here, continuous wave and pulsed microwave and radio frequency plasmas in nitrogen-containing gas mixtures were studied for grafting of nitrogen functional groups on polystyrene. Plasma conditions were optimized in two respects: either to obtain a high selectivity for amino groups, or to maximize the overall density of nitrogen groups. The obtained functionalized surfaces were investigated by means of XPS, contact angles and cell culture. Specific plasma conditions lead to surfaces with high-density cultures of adherent cells after 24 hours of culturing, exceeding significantly densities on the untreated or oxygen-plasma-treated polymer. The highest level of nitrogen and amino functionalization was obtained using pulsed microwave plasmas. Patterning of the chemical functionalization was realized by a hydrogen plasma treatment using a laser-cut metal mask. The chemical pattern was verified by XPS with high local resolution.

4:40pm **BI-ThA9 Controlled Cell Adhesion on Honeycomb Films of Biodegradable Polymers**, ***T. Nishikawa,*** RIKEN Frontier Research System, Japan*; K. Nishikawa, R. Ookura, J. Nishida,* Hokkaido University, Japan*; K. Arai, J. Hayashi,* RIKEN Frontier Research System, Japan*; M. Matsushita, S. Todo,* Hokkaido University, Japan*; M. Hara, M. Shimomura,* RIKEN Frontier Research System, Japan

We report that a honeycomb like micro-porous film (honeycomb film) can control cell adhesion of hepatocytes and cardiac myocytes. The honeycomb films were fabricated by casting a dilute solution containing biodegradable polymers (poly-L-lactic acid (PLLA) and poly-@epsilon@-caprolactone (PCL)) and an amphiphilic polymer on water surface in a humid atmosphere. By the method, self-supported honeycomb films were obtained. Hepatocytes were cultured on a self-supported honeycomb film of PLLA. The cells formed a single layer of columnar shape cells with a thickness of 20 µm. The tissue formation of hepatocytes specifically occurred on the honeycomb films of PLLA, but not on flat films of PLLA. The artificial tissue of hepatocyets expressed high level of albumin secretion, which was comparable to that of spheroids of hepatocytes. Furthermore we succeeded in three dimensional culturing of hepatocytes. Hepatocytes formed two single layers on each sides of a self-supported honeycomb film of PLLA. Honeycomb film of PCL was stretched out uniaxially by mechanical force. The honeycomb pores were deformed into elongated hexagons and rectangles. Since the array of the elongated hexagons is anisotropic, the stretched honeycomb film is applicable to guiding cell alignment. We used a stretched honeycomb film of PCL as a cell culture substrate for cardiac myocytes. The substrate was fabricated by placing a stretched honeycomb film of PCL onto a glass plate. Cardiac myocytes of rat embryo were not aligned in a specific direction on regular honeycomb patterned surface. On the other hand, cardiac myocytes were aligned along the long axis of the stretched micro-pores on the stretched honeycomb film. Thus the honeycomb films can control cell alignment as well as cell attachment. Based on the results, we expect that the honeycomb films can be designed, fabricated, and utilized in accordance with target tissues.

5:00pm **BI-ThA10 New Substrates for Retinal Cell Transplantation**, ***C.J. Lee****, S.F. Bent, P. Huie, M. Blumenkranz, H. Fishman,* Stanford University

A novel treatment for age related macular degeneration (AMD) is currently being investigated. This treatment involves the transplantation of human pigment epithelial cells (PE) on a carrier substrate to rescue the diseased retina. Various substrates including synthetic biodegradable polymers and biocompatible substances have been proposed as carrier substrates. Biocompatible materials offer the ability to coexist in the subretinal space, thus reducing immune rejection. The goal of this work was to grow cells on various biocompatible materials and to show the survival and longevity of the cells. Without specific constraints, the cells exhibit a variety of morphologies, including cuboidal and elliptical structures. In this study, surface modifications were employed to control the growth and morphologies of the cells. The cells have been successfully grown on these modified substrates, exhibiting stable function for at least two weeks in culture. In summary, we show that engineering biocompatible substrates is possible and that stable growth of cells occurs. The possibility of the feasibility of this treatment in animals will be discussed.

Biomaterials

Room 134/135 - Session BI-ThP

Biomolecule and Cell Poster Session

BI-ThP1 Interactions of Dye Molecules with Poly(Propylene Imine) Dendrimer Monolayers, *S. Chen, L. Li, C. Boozer,* ***S. Jiang,*** University of Washington

Dendrimers have been regarded as interesting candidates for applications in host-guest chemistry, such as complex drug delivery agents. A fundamental understanding of guest-host interactions in dendrimers will facilitate the effort to design new-generation chemical and biological sensors based on dendrimers. In this work, we report a systematic study of interactions between a group of dye molecules and poly(propylene imine) dendrimers (PPI) tethered to carboxyl-terminated self-assembled monolayers (SAMs). Adsorption kinetics is measured by surface plasma resonance (SPR) sensors. The fifth-generation PPI dendrimers are mainly used in this work. Our results show a strong affinity of PPI-5 to Rose Bengal or erythrosine B. The strong affinity results from well matches in charge and structure between the guest and the host molecules. In addition, the effect of various factors (e.g., dendrimer generations, charges and shapes of guest molecules, and pH values and ionic strengths of liquid solutions) on adsorption will be discussed. This is the first attempt to study the adsorption of small molecules using SPR.

BI-ThP2 Protein Adsorption on Mixed Self-assembled Monolayers, ***L. Li***, *S. Chen, C. Boozer, S. Jiang,* University of Washington

Mixed self-assembled monolayers (SAMs) of alkanethiols on Au(111) can be used to precisely control molecular-scale chemical, structural, and biological surface properties via controlling the abundance, the type, and the spatial (both normal and lateral) distribution of tail group sites. By controlling surface microenvironment, different structures and activities of immobilized proteins are expected. Here, we first report our recent studies on phase behavior of mixed alkanethiols with two compounds having different chain lengths (C8-C18) and terminal groups (-COOH, -OH, -CH@sub 3@, and -NH@sub 2@) on Au(111). These mixed SAMs are characterized by scanning tunneling microscopy (STM) and atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), contact angle measurements, and ellipsometry. Results show that closely packed and homogeneously mixed SAMs can be achieved at the molecular level. These controlled surfaces will then be used for the adsorption of various proteins such as albumin, lysozyme, etc. Adsorption behavior is characterized by tapping-mode AFM and surface plasmon resonance (SPR) sensors. Results show that molecular-scale mixed SAMs generally promote protein adsorption. The effect of the abundance, type, and spatial distribution of terminal groups on protein adsorption is explored systematically in this work. Keywords: Mixed SAMs, protein adsorption, AFM, SPR, and XPS.

BI-ThP3 Studies of Phosphopeptides on Metal Impregnated Plasma Polymer Surfaces, ***J. Zhang***, *J.D. Whittle, H. Qiu, R.B. Timmons, G.R. Kinsel,* The University of Texas at Arlington

Surface-protein interactions play an important role in the fields of biology and medicine. Recent work in our group has focused on the binding affinity of phosphopeptides for metal ions immobilized on vinyl acetic acid modified PET substrates. Our work demonstrates that, under specific solution conditions, phosphopeptides have high binding affinities for copper. This observation has been utilized to purify / clean-up phosphopeptides on-probe before sample analysis by Matrix Assisted Laser Desorption / Ionization (MALDI) Mass Spectrometry (MS). Our initial studies focus on the development of surfaces for extraction / isolation of phosphopeptides via coordination with surface bound metal ions. PET substrates (4.8 mm diameter disks) were modified by pulsed RF plasma deposition of polymerized vinyl acetic acid. Metal ions were incorporated into the vinyl acetic acid modified PET substrates by immersion of the substrates into various metal ion solutions. Specific binding of phosphopeptides to the surface was first demonstrated by exposure of the metal impregnated film to a mixture of the peptide buccalin and the phosphopeptide p60 substrate II. Washing of the surface with the buffer MES (pH = 5.5)/10%acetonitrile led to selective removal of the buccalin peptide from the surface film. (This step was confirmed by MALDI analysis of the wash solution.) Similar methods have also been successfully applied to the extraction of phosphopeptides from alpha-casein tryptic digests. The purification of phophopeptides resulted in an increase in the peptide MALDI ion signal and improved ion signal resolution. Additional studies have focused on the effect of changes in the metal ion used for phosphopeptide binding, changes in the solutions used for washing / peptide release, and metal-ion binding of histidine-rich peptides.

BI-ThP5 Time-of-flight Secondary Ion Mass Spectrometry (ToF-SIMS) Characterization of Adsorbed Proteins with Amino Acid Mutations, ***N. Xia***, *R. To, S.L. McArthur, P.S. Stayton, D.G. Castner,* University of Washington

Static time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to detect amino acid sequence mutations in adsorbed streptavidin. A series of mutations were introduced into wild-type streptavidin using site-directed mutagenesis. Both wild-type and mutant strepavidin were adsorbed onto polystyrene. Then positive and negative static ToF-SIMS spectra were acquired. For mutants where one amino acid (either glutamic acid or serine) in each strepavidin monomer was replaced with a cysteine the largest differences were observed in the negative spectra. The appearance of ToF-SIMS peaks at 32 (S@super -@) and 33 (HS @super -@) indicated the incorporation of cysteine into the streptavidin mutants. Only small changes in the positive ion ToF-SIMS spectra were observed upon incorporation of cysteine into streptavidin (e.g., the appearance of a small peak at 45 due to the CHS @super +@ fragment from cysteine). Introduction of a larger mutation (linking a histidine tag to the C-terminus of each streptavidin monomer) resulted in larger changes in the positive ion ToF-SIMS spectra. Due to the relative complexity of the positive spectra from proteins, principal component analysis (PCA) was used to analyze the differences in the positive spectra. The largest changes introduced by the presence of the histidine tags were observed in the intensities of the peaks at m/z=81, 82 and 110, which correspond to the primary mass fragments from the amino acid histidine.

BI-ThP7 Dynamics and Chemistry of ELISA Test for Toxins by In-situ and Ex-situ Spectroscopic Ellipsometry, ***D.W. Thompson***, *E.M. Berberov, G.L. Pfeiffer, J.A. Woollam,* University of Nebraska-Lincoln*; T.E. Tiwald, C.M. Herzinger,* J.A. Woollam Co., Inc.*; T. Datta,* University of South Carolina

Fast, in-situ spectroscopic ellipsometry at 225 simultaneous visible wavelengths is used to study dynamics of deposition in liquid solution of each layer in a four-layer enzyme-linked immunosorbent assay (ELISA) for cholera toxin. Time-dependent functional relationships of cholera and Escherichia coli heat-labile enterotoxin attachment to monosialoganglioside (G@subM1@) coated substrates are studied, as well as the attachment of associated antibodies. Ellipsometric selectivity between toxins is demonstrated. In addition, ex-situ spectroscopic ellipsometry from vacuum-ultraviolet (131 nm / 9.5 eV) to mid-infrared (35 microns) on each constituent layer of the ELISA is studied. Several distinct oscillator-like features in the visible to vacuum-UV are found at 1.42 eV, 4.24 eV, 6.32-6.35 eV, and 10.98 eV. Protein resonant chemical oscillators in the individual layers are identified as: C-H in CH@sub2@ and CH@sub3@, N-H, O-H, and P=O, seen at 3307 cm@super-1@, 2926 cm@super-1@, 2958 cm@super-1@, 1662 cm@super-1@, 1546 cm@super-1@, 1245 cm@super-1@ and 1081 cm@super-1@. In summary, in-situ and ex-situ spectroscopic ellipsometry covering vacuum-UV to mid-infrared is a simple, nondestructive way to study dynamics and chemistry of nanometer dimension ELISA films. Research supported by NSF SBIR contract number NSF II-9901510.

BI-ThP8 Do the Chemical Properties of Polymeric Surfaces Influence the Bacterial Adhesion?, ***G. Speranza***, *R. Canteri, C. Pederzolli, G. Gottardi, L. Pasquardini, E. Carli, M. Grosello, A. Lui, M. Anderle,* ITC-irst, Centro per la Ricerca Scientifica e Tecnologica, Italy

Understanding the mechanisms underlying the interactions between biological systems and polymeric surfaces is of paramount importance. In fact, for their physico-chemical properties, polymers as biomaterials, are utilized in a even larger variety of applications. However, an increasing significance in the medical routine is assumed by the development of bacterial infections caused by implanted polymeric devices (up to 40% of nosocomial infections). In this respect the investigation of the phenomena which take place at the interface between polymeric surface and bacterial wall, are gaining a great relevance. Due to the high level of complexity, these processes, to the best of our knowledge, are still not well clarified. Aim of this work is to investigate this topic to reach a deeper degree of comprehension. The description of the interfacial interactions at the biomaterial surface normally make use of the van der Waals forces. A new term that involves acid-base interactions is here hypothesized to fully describe the bacterial adhesion to the polymer surface. Two requirements are needed to test this hypothesis: an ideal polymeric surface in terms of chemical and morphological properties and "standard samples" as bacterial strains. Several experiments were worked out using the Escherichia Coli (Gram-) strain and its growth on polymers having an "acid" or "basic"

character, was investigated. The first findings seems to indicate that the bacterial adhesion is influenced by the chemical properties of the polymeric surface. This first results may be interpreted taking into account a mechanism in which the acid/base (Lewis) interaction plays an important role.

BI-ThP9 Photoluminescent Study of Bacterial Lipid A Langmuir-Blodgett Monolayers, ***V.V. Kislyuk***, *L.D. Varbanets, Z.I. Kazantseva, I.M. Pahuta, V.Z. Lozovski,* National Academy of Sciences of Ukraine, Ukraine

The photo luminescent spectra have been studied at room temperature for the Lipid A Langmuir-Blodgett monolayers grown on various substrates of Au, Si and SiO@sub 2@. The maxima of the spectral profiles are shifted towards long waves as compared to the spectra of 10@super -2@% DMSO solution and water suspension of the lipid. The energy difference of approximately 0.4 eV is, likely, attributed to the molecule-molecule and molecule-surface interaction of the monolayer. The luminescence has been stimulated with pulses of UV laser (337 nm, 100 Hz, 8 ns) or UV lines of Xe lamp and registered with photon counter with the coincidence circuit. The lipid A were extracted from the membrane of Ralstonia Solancearum.

BI-ThP10 Human Foreskin Fibroblast Cell Studies on Micropatterned Substrates Created by Selective Molecular Assembly Patterning, ***J.W. Lussi***, *R. Michel, A. Goessl, M. Textor, J.A Hubbell,* Swiss Federal Institute of Technology, Switzerland

A novel patterning technique, termed "Selective Molecular Assembly Patterning" (SMAP), was used to produce cell-adhesive patches on a cell-resistant background. The method is based on selective adsorption of alkane phosphates to titanium oxide, but not silicon oxide surfaces. Using standard photolithographic techniques patterns of titanium oxide within a matrix of silicon oxide were created. A self-assembled monolayer of dodecyl phosphate (DDP) formed on the titanium oxide, while poly(-L-lysine)-poly(ethylene glycol) graft copolymer (PLL-PEG) subsequently rendered the silicon oxide matrix resistant to cell adhesion. A combination of phase contrast and fluorescent microscopy was used to examine the spreading of human foreskin fibroblast (HFF) cells seeded on the patterned substrates. Unstained cells, as well as cells stained for cytoskeletal f-actin and the focal adhesion protein vinculin, were examined. HFFs adhered only to the DDP-coated TiO2 patches, clearly recognizing the substrate pattern. The PLL-PEG coated SiO2 matrix remained completely resistant to cell adhesion in fetal bovine serum containing medium for more than 5 days. Cell surface contacts were observed on DDP-coated TiO2 patches and stress fibers traversed patterned features. Cell shape on patterned substrates differed significantly from HFF cultured on homogenously cell-adhesive substrates. Cells exhibited more angular shapes imposed by the square symmetry of the oxide pattern. Cell extensions frequently bridged several features without establishing contacts to the background surface. These cell experiments conclusively demonstrate the usefulness of this patterning method for studying cell adhesion on patterned surfaces.

BI-ThP11 Self-Assembly Approaches to Preparation of Self-Supported Porous Films for Selective Cell Separations and Tissue Engineering Scaffolds, ***M. Tanaka***, *M. Miyama, K. Nishikawa, J. Nishida, M. Shimomura,* Hokkaido University, Japan

Selective cell separation is one of the most important issues in the field of tissue regenerative medicine at present. We prepared self-supported porous films of poly(@beta@caprolactone) or poly(lactic acid-co-glicolic acid) with wide range of controlled pore size (0.5-100 µm) and area (diameter 1-20 cm) to develop high-performance filters and tissue engineering scaffolds. The self-supported porous films were fabricated by a simple casting technique and self-assembly proceses@footnote 1,2@ without lithographic methods. The films had highly regular patterns and stable net structure with high mechanical stability and moderate elasticity. Creation of desirable pore size was easy, and the pore size can be controlled simply by changing the evaporation rate of the polymer solutions. The porous film which had been a novel biocompatible poly(2-methoxyethyl acrylate)@footnote 3,4@ coated was attached to a module having an inlet port and outlet port to simulate a leukocyte eliminating. The film provided the 100% selective separation of the leukocyte from human blood. The leukocyte eliminating ratio and platelet recovery were dependent on the pore structures (size and depth) and the chemical properties (hydrophilicity and surface charge). The biocompatible and biodegradable films could be expected as promising materials to produce useful selective cell separations, implants and cell-supported elastic scaffold for various medical devices. @FootnoteText@@footnote 1@N. Maruyama et al., Thin Solid Films, 327-329, 854-856 (1998) @footnote 2@T. Nishikawa et al., Mater. Sci. & Eng. C, 10, 141-146 (1999) @footnote 3@M. Tanaka et al., Biomaterials, 21, 1471-1481 (2000) @footnote 4@M. Tanaka et al., Polymer Int, 49, 1709-1713 (2000)

BI-ThP13 Analysis of Cell Organization in a Smooth Muscle Culture Grown On High Aspect Ratio Microstructures, ***J.D. Glawe***, *D.K. Mills, B.K. Gale,* Louisiana Tech University

Tissue engineered organs may someday replace donated organs. The first step towards realizing this goal is to create three dimensional cell cultures that preserve the organization of cellular orientation found in tissue in vivo. One promising method of achieving this goal is to microfabricate scaffolds with microcontainers designed to allow cell growth in only one direction. This is essential for engineering muscle tissue where to remain functional, all cells must contract along the same axis. The tissue culture scaffold was specifically designed to accommodate smooth muscle cells (SMC's). It was molded out of polydimethylsiloxane (PDMS) from a lithographically patterned SU-8 structure. The structure is coated with protein to promote improved cell adhesion. Two methods of seeding the cells into the scaffold were tested, gravitational and centrifugal seeding. The microcontainers are approximately 500 µm in depth and are either square, octagonal, or plus-shaped, with varying diameters. Measurements were made to determine cell density, orientation, actin concentration, and the percent of living cells at four intervals after cell seeding.

BI-ThP14 Ion Implanted Titanium: Relating Surface Chemistry to Cellular Response., ***F.H. Jones***, *L. Shinawi, S. Nayab, I. Olsen, J.A. Hobkirk,* University College London, UK; *T.J. Tate, D.S. McPhail,* Imperial College of Science, Technology and Medicine, UK

Titanium-based materials are used extensively in hard tissue biomedical implants, often with inorganic coatings to promote bone regeneration and integration. The properties of such coatings remain less than ideal; variable composition, dissolution at low pH and fracture failure or delamination continue to present serious problems. Direct modification of the surface using ion implantation has been proposed as a possible alternative, giving a favourable interface for interaction with the host tissue, without affecting bulk properties. The homogeneity and controllability of ion implantation also mean that the technique is ideal for studying the fundamental effects of chemical composition on cellular response to a surface. Ca implantation has been shown to promote osseointegration,@footnote 1@ but little attention has been paid to the effect of the nature of the substrate on the resulting surface chemistry, or the difference in behaviour of surfaces implanted with different ions. The current work examines the effect of ion implantation into native Ti, air oxidised Ti and TiO@sub 2@ single crystals. Ca, K and Ar ions, selected due to their similar masses, were implanted at doses up to 2x10@super 17@ ions cm@super -2@. Preliminary cell culture studies indicate significant differences in cell behaviour depending on the chemical nature of the implanted ion. Of particular interest are adverse effects observed on Ar-implanted surfaces, despite the inert nature of argon. In parallel, XPS and SIMS were used to investigate surface chemistry. The effects of annealing in UHV and immersion in water were found to be element-dependent. The nature of the ion was also found to influence the interaction with model organic species in solution and the rate of calcium phosphate deposition from mineralising solutions. @FootnoteText@ @footnote 1@ Hanawa T, Kamiura Y, Yamamoto S, Kohgo T, Amemiya A, Ukai H, Murakami K, Asaoka K. J. Biomed. Mater. Res. 36 (1997) 131.

Biomaterials
Room 102 - Session BI-FrM

Biosensors
Moderator: A.T.A. Jenkins, University of Bath, U.K.

8:20am **BI-FrM1 Planar Membrane Assemblies and Waveguide Optics for Biomolecular Device Applications**, ***S. Saavedra***, University of Arizona **INVITED**

This talk will focus on creation and characterization of supramolecular architectures, composed of polymerized lipid bilayer membranes functionalized with proteins, for use in sensor transduction. Novel optical transduction techniques based on planar waveguide optics will also be discussed.

9:00am **BI-FrM3 TOF-SIMS Analysis of Nucleic Acid Biosensor Chips**, ***H.F. Arlinghaus***, *M. Ostrop, O. Friedrichs, J.C. Feldner,* Universität Münster, Germany

In recent years DNA-chip-technology has been a subject of growing interest for clinical diagnostics as well as for DNA sequencing and forensic. DNA-chips are based on the method of sequencing by hybridization (SBH), where unknown DNA fragments are hybridized to complementary oligodeoxynucleotides (ODN) which are immobilized on a solid surface in an array format. The main variables in SBH are the attachment of the nucleic acid sequences to a solid surface, the conditions for hybridization, and the detection of the hybridized DNA sequences. We have used TOF-SIMS to examine in detail the immobilization process of PNA/ODN and to investigate its ability to detect DNA-fragments hybridized to complementary PNAs. For this purpose we immobilized either silane SA-layers on silicon wafers or DTSP SA-layer on Ag or Au surfaces. PNA and DNA of different concentrations were then bound to these layers. Deprotenated (M-H)@super -@ signal of the different ODN and PNA bases as well as phosphate ions were used to monitor the ODN/PNA concentration. It was found that the immobilization process is strongly dependent on concentration and immobilization time. Under optimized conditions, PNA can be covalently bound to these surfaces. The homogeneity of the immobilized PNA depends on the evaporation time of the PNA solution. After optimizing the immobilization process, complementary and non-complementary DNAs were hybridized to the PNA biosensor chip. Hybridized DNA could be readily identified by detecting the PO@sub 2@@super -@ and PO@sub 3@@super -@ masses. A good discrimination between complementary and non-complementary sequences could be achieved. It can be concluded that TOF-SIMS is a very useful techniques for investigating the complexity of the immobilization and hybridization processes and that SIMS has the potential for providing a rapid method for DNA/RNA sequencing and diagnostics. .

9:20am **BI-FrM4 A "Label-Free" Microchip Array Based Protein-Binding Assay using Surface Plasmon Resonance Imaging**, ***C.E.J. Dentinger***, *D. Martin, P. Wagner,* Zyomyx, Inc.

We demonstrate a microchip array based antibody binding assay that relies on imaging surface plasmon resonance (SPR) for detection of protein binding. Since SPR is sensitive to the index of refraction near a gold surface this assay does not require the binding proteins to be labeled (e.g. fluorescently or with a radio label), making it a "label free" detection technique. For our imaging SPR assay we have developed a method of specifically immobilizing proteins on surfaces that resist non-specific protein adsorption. These surfaces consist of a gold substrate coated with an omega functionalized alkanethiol self-assembled monolayer (asymNHS). The succinimidic ester head group of this monolayer is then reacted with biotin-LC-PEO-amine to make a biotin-coated surface. This surface will bind streptavidin in a manner that leaves some of streptavidin's binding pockets open to immobilize a wide variety of biotinylated proteins. The asymNHS, biotin-LC-PEO-amine, and streptavidin layers are characterized with ellipsometry, and Fourier transform infrared reflection adsorption spectroscopy (FTIRRAS). The "label-free" assay is then performed by immobilizing biotinylated proteins in an array format and monitoring the different antibody-antigen interactions on this array by imaging SPR. We have shown that this surface will bind a second protein only to the areas where the biotinylated proteins are immobilized. We have also demonstrated that with our imaging SPR we have the ability to watch the binding assays in real time.

9:40am **BI-FrM5 Comparative Analysis of Immobilization Strategies for Antibodies and Their Fragments on Biosensor Surfaces**, ***P. Peluso***, *D. Wilson, D. Do, M. Venkatasubbaiah, H. Tran, P. Kernen, K. Witte, B. Heidecker, P. Wagner, S. Nock,* Zyomyx, Inc.

Advancement in the study of proteomics requires the development of microarray biosensors which possess a high degree of sensitivity. Towards this goal, strategies must be optimized to couple biomolecular capture agents to matrices at sufficient densities without compromising their binding properties. Using SPR and radioimmunoassay methods, we have examined the effect of different coupling chemistries on the binding activities of various antibodies and their corresponding Fab forms. Novel methods for attaching antibodies and Fab fragments to surfaces were employed and compared with random coupling procedures. In general, we found oriented attachment to better maintain the binding activity of these molecules. Moreover, oriented-Fab surfaces appear to exhibit the highest molecular density and binding activity. We further extrapolated these findings to performance within a microarray format utilizing a fluorescence-based sandwich assay.

10:00am **BI-FrM6 Electrostatically Assisted SPR: A New Method for DNA Mutation Detection**, ***L.K. Wolf***, *R.J. Heaton, A.W. Peterson,* Boston University*; R.M. Georgiadis,* Boston University, usa

Detection of DNA base-pair mutations is essential for the study and treatment of genetic disorders and disease. Present-day studies use DNA microarrays and fluorescently labeled probes for high-throughput mutation analysis. Recently, we have discriminated between matched and mismatched surface immobilized duplexes by evaluating surface coverage and hybridization kinetics with electrostatically assisted surface plasmon resonance (SPR).@footnote 1@ Here, we show that this method allows label-free in-situ detection of a single A-C base-pair mismatch in a 25-mer oligonucleotide duplex. We also apply electrostatically assisted SPR to other types of mismatches, demonstrating the generality and usefulness of this method to mutation detection. The effects of ionic strength on mismatch discrimination are also investigated. @FootnoteText@ @footnote 1@ R. J. Heaton, A. W. Peterson, and R. M. Georgiadis, Proc. Nat. Acad. Sci. 98, 3701 (2001).

10:20am **BI-FrM7 DNA Assay Sensitivity in the BARC Magnetoresistive Biosensor**, ***M.A. Piani***, NOVA Research, Inc.*; R.J. Colton, M. Miller, J.C. Rife, P.E. Sheehan,* Naval Research Laboratory*; C.R. Tamanaha,* Geo-Centers, Inc.*; L.J. Whitman,* Naval Research Laboratory

The Bead ARray Counter (BARC) biosensor uses DNA microarrays, magnetic microbeads, and giant magnetoresistive (GMR) magnetic field sensors to detect and identify biological molecules.@footnote 1@ The core of the sensor is a small, microfabricated chip containing an array of 64 GMR sensors. Distinct single stranded DNA capture probes are immobilized on each sensor. Complementary DNA in a sample is allowed to hybridize on the chip, and is then labeled with magnetic microbeads that are detected by the GMR sensors. The overall system sensitivity is a convolution of the chemical and GMR sensor sensitivities. The chemical sensitivity is determined by the effectiveness of the hybridization and labeling assay. Our current chemical sensitivity is 10 fM for a 60 minute hybridization of 30 bp oligonucleotides. Our efforts to improve this sensitivity involves two tracks. First, we are working with chemiluminescent assays in order to optimize the immobilization and hybridization steps. Second, we are experimenting with peptide nucleic acid (PNA) capture probes instead of DNA. PNA is a synthetic oligonucleotide analog with a neutral backbone that hybridizes with complementary DNA with a higher affinity then the DNA complement. Furthermore, PNA molecules are resistant to nucleases ("DNAses"), enabling the BARC chip to be reconstituted and reused following treatment with the enzyme. Our initial results with PNA indicate an increase in chemical sensitivity of one to two orders of magnitude. @FootnoteText@ @footnote 1@Edelstein et al., Biosensors & Bioelectronics 14, 805 (2000).

10:40am **BI-FrM8 Study of the Formation and Function of the Cell Membrane Hybrid Layers Containing G-Protein Coupled Receptors at SAM Surfaces**, ***V. Silin***, National Institute of Standards and Technology*; R. Madhusudhana,* Institute of Biochemical Research, India*; J.T. Woodward, K.D. Ridge, A. Plant,* National Institute of Standards and Technology

Cell membrane vesicles can reorganize at an alkanethiol/lipid mixed monolayers or alkanethiol monolayer-coated surface resulting in the formation of cell membrane hybrid (CMH) layers. The ability to form CMH layers at the surface from various cell types expressing G-protein coupled receptors offers a promising method for the rapid screening of potential membrane receptor ligands. In our work CMH layers were formed using

membrane vesicles from monkey kidney COS-1 cells that had been transiently transfected with synthetic human CCR5 chemokine receptors. CMHs were formed on a thiahexa-(ethylene oxide)-octadecane (THEO-C18) thiol surface or on a mixed POPC/THEO-C18 surface. AFM and SPR technique showed that the membrane form continuous layers at the surfaces with a thickness around 4 to 5nm for different samples. CMH surfaces were tested using specific antibodies against CCR5 receptors to check the surface concentration of the receptors at the surfaces and their orientation. The specific binding of the chemokine ligand was detected at this surface over different concentration. The kinetics of the CMH formation, as well as antibody and ligand binding to the surfaces was measured using the SPR technique, which showed extremely good sensitivity for this application. A number of control experiments were carried out with nonspecific antibodies, ligands and membranes to support data for the specificity of the CCR5 receptor response.

11:00am **BI-FrM9 Surface Chemistry for a Membrane-based Biosensor@footnote 1@**, ***C. Cole***, Nova Research, Inc.*; M. Natesan,* Geo-Centers, Inc.*; M. Malito,* Nova Research, Inc.*; R.J. Colton, L.J. Whitman,* Naval Research Laboratory

A multianalyte biosensor has been developed that uses magnetic force to differentiate between specific and nonspecific ligand-receptor/ligand-surface interactions.@footnote 2@ The initial sensor was developed as a 36-well microtitre plate, using surface chemistries developed to modify polystyrene.@footnote 3@ In order to enhance sensitivity, reduce assay time, and increase portability, a second generation sensor is being developed that replaces the polystyrene with an alumina ultrafiltration membrane. Although the use of inorganic supports is not necessarily novel, the application of polymer coatings designed to reject protein adsorption to such a surface is. We have developed methods to introduce desired surface functionalities onto commercial membranes. At the same time, we have addressed the potentially more critical issue of ensuring that our coatings are highly reproducible, thus providing for a biosensor with consistent response. To supplement XPS, semi-quantitative methods have been developed to specifically determine the extent of poly(ethyleneimine) and poly(ethyleneglycol) coverage; resulting lots of derivatized surfaces have been found to be indistinguishable in assay use. The membrane-based biosensor has been used to detect proteins, bacteria, and viral particles in 25 minutes or less, with an enhancement in sensitivity of 2-3 orders of magnitude compared to the initial prototype (e.g., 10@super 3@ pfu/mL MS2 vs. 10@super 5@ pfu/mL on the plate-based sensor). Various arraying technologies are being evaluated, including photolithography with activated biotin, mechanical masking, and arraying through site-specific chemistry. Use of these technologies to produce an arrayed filter for multianalyte detection will be discussed. @FootnoteText@ @footnote 1@ Supported by the Joint Technical Panel for Chemical and Biological Defense @footnote 2@ Lee et al., Anal. Biochem. 287, 261 (2000) @footnote 3@ Metzger et al., J. Vac. Sci. Technol. A 17, 2623 (1999).

11:20am **BI-FrM10 An Electroactive Substrate for Direct Transduction of Biorecognition**, ***J. Yan***, *P. Hampton, G. López,* The University of New Mexico

Direct electrochemical transduction of biorecognition on well-defined and protein-resistant electrode surfaces has recently attracted enormous attention. Herein, we report the design and characterization of self-assembled monolayers (SAMs) presenting protein binding ligands, electroactive probe molecules and protein-resistant oligo(ethylene glycol) moieties that allow direct electrochemical transduction of biorecognition at modified electrodes. The electroactive compound, N-(11-mercaptoundecyl)-N'-carboxymethyl-4,4'-bipyridinium dibromide (1), was selected and synthesized because of the known environmental sensitivity and reversibility of the redox chemistry of the viologen groups (i.e., 4,4'-bipyridyl dications) and potential to derivatize the acid terminus with biological molecules. Mixed SAMs of compound 1 and (1-mercaptoundec-11-yl)tri(ethylene glycol) (2) on gold were prepared by coadsorption from a solution of 1 and 2 (@chi@@sub 2@ = 0.6). N-(5-aminopentyl)biotinamide was subsequently attached to the surface through amide bonds. Cyclic voltammetric, ellipsometric and X-ray photoelectron spectroscopic measurements confirmed the attachment of the biotin ligands and the efficiency of the SAM in preventing nonspecific protein binding. Incubation of the biotin-modified SAM in a phosphate buffered saline (PBS, pH 7.4) containing 30 µg/ml anti-biotin for 2 h resulted in a significant negative shift in the redox potential of viologen moieties and a decrease in the peak currents and the charging currents. Incubation of the biotin-modified SAM in PBS containing biotin-blocked anti-biotin for 2 h, however, showed no change in the redox potential, peak currents and charging currents, indicating that the adsorption was biospecific.

11:40am **BI-FrM11 Crystalline Bacterial S-Layer Proteins: A New Supporting Structure to Separate and Stabilize Lipid Membranes**, ***B. Schuster***, *P.C. Gufler, D. Pum, U.B. Sleytr,* Universitaet fuer Bodenkultur Wien, Austria

A key component in the combination of membrane-associated molecular recognition mechanisms with inorganic surfaces is an ultrathin layer separating the lipid membrane and the solid surface. The demands on this layer are manifold as it should both, stabilize and maintain the fluidity and structural properties of the lipid membrane. This is an essential feature providing an environment for reconstitution and immobilization of membrane proteins under non-denaturing conditions. One promising strategy is the application of bacterial cell surface layers (S-layers) to support (bilayer) lipid membranes (BLMs). S-layer proteins are the simplest self-assembly systems that produce crystalline, nanometer-thick, isoporous lattices with well-defined topographical and physico-chemical properties. S-layer proteins have been recrystallized on solid supports like gold or silicon wafers, or deposited on porous polymer filters. Attached BLMs exhibit an increased fluidity compared to dextran- or silane-supported BLMs and the stability is significantly enhanced. By contrast with the less stable BLM on polymer filters, successful reconstitution of staphylococcal alpha-hemolysin was observed with BLMs separated by an S-layer from the porous support. The unitary conductance of a hemolysin pore was found to be similar reconstituted in S-layer supported BLMs on porous supports and in common folded BLMs. As an alternative to soft polymer cushions, to hybrid or tethered lipid membranes, S-layer supported lipid membranes provide a biomimetic, water-containing environment for transmembrane proteins. Furthermore, composite S-layer/lipid membranes in combination with new sensor technology might play an important role in the development of biosensors.

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