Biomaterials
Room: C-201 - Session BI-MoM

Theoretical Studies of Biosurfaces/Biotribology and Biorheology
Moderator: R.A. Latour, Clemson University

9:00am **BI-MoM3 Molecular Simulation Studies of Orientation of Antibodies Adsorbed on Charged Surfaces**, *J. Zhou, J. Zheng,* ***S. Jiang***, University of Washington

Antibodies have found many applications in biotechnology and clinical medicine, including diagnostic assays, environmental testing, and process monitoring. It is well-known that Fab fragment of an antibody can bind its antigen with a very high specificity. Therefore, it is desirable to control the antibody orientation for immunoassay applications. In this work, Monte Carlo simulations are performed to study and predict the adsorption and orientation of antibodies as a function of surface and solution properties using hierarchical models, a simplified Y-shape 12-bead model antibody, a united-residue model, and an all-atom model. For all these three models, simulation results show that higher surface charge density and lower solution ionic strength favor narrower orientation distribution of adsorbed antibodies. Simulation results further show preferred antibody orientation under controlled surface and solution conditions, which are verified by our SPR and ToF-SIMS experiments. For the 12-bead model, it allows us to quickly map out the general trends of the orientation behavior of antibodies on surfaces. For the residue model we developed, more detailed residue-distribution information of antibody near surfaces can be achieved. For the all-atom model, the conformation change of an adsorbed antibody was obtained with a proposed hybrid method. The fundamental understanding of antibodies on surfaces of this work will facilitate the effort to develop better biosensors.

9:20am **BI-MoM4 Mapping the Free Energy State of Water in Hydration Layers and Its Importance for Ligand-Receptor Binding**, ***G.W. Grahek***, *R.A. Latour, S.J. Stuart*, Clemson University

The thermodynamic contributions of solvent molecules during ligand-receptor binding are generally believed to be very important, but relatively little is actually understood regarding how the entropy, enthalpy, and free energy of hydration layers change as a ligand approaches and docks with its receptor. We assume that both ligands and receptors perturb the thermodynamic state of their localized hydration layers, and that these effects must be superimposed on the intervening water layers as a ligand approaches its receptor. It is hypothesized that this effect may have a significant influence on the height of the activation barrier for ligand-receptor binding, and thus may serve as an important medium-range modulator of ligand-receptor binding. Based on this underlying hypothesis, we are investigating the development of statistical mechanics based molecular modeling methods to calculate the entropy, enthalpy, and free energy values of water as a function of position surrounding a designated solute molecule. Simulations have been conducted using both molecular dynamics (AMBER 6.0) and Metropolis Monte Carlo (BOSS 4.2; OPLS) methods using TIP3P and TIP5P water, respectively, and periodic boundary conditions surrounding a centralized solute molecule. Entropy, enthalpy and free energy are then mapped on a 3-dimensional grid. The simulations indicate that distinct changes do occur in the calculated free energy state of water molecules in the first two hydration layers surrounding the solute compared to bulk water. Further studies are being planned to investigate the effect of solute-solute separation distance on the intervening water layers. Following final development, these methods will be applied to actual ligand-receptor systems for the purpose of predicting the influence of water structure on binding. It is believed that these simulations may provide new insights that will facilitate drug design for specific receptor targets.

9:40am **BI-MoM5 Growth of a Polymer Brush from Solution: Adsorption, Desorption, Conformational Conversion and Charging**, ***H.J. Kreuzer***, Dalhousie University, Canada **INVITED**

Going beyond mean field theory we develop a model of a polymer brush that allows for inhomogeneity, confinement and lateral interactions. The model is developed for freely rotating chains and a realistic Interacting Chain Model for poly(ethylene glycol). The parameters in the latter are obtained from a first principles theory based on (i) ab initio (density functional theory) calculations of the potential energy surfaces of the polymer conformers, and (2) the proper statistical mechanics for which we succeeded to formulate and solve a Green's function approach (transfer matrix method) in the presence of an external force field. We set up kinetic equations for the time evolution of the growth of a brush from solution. For PEG a detailed analysis and discussion of recent data is made that identifies two time regimes of pancake adsorption and collision-induced conformational conversion to stretched moeities, respectively. Lastly we discuss the possibility that auto-ionization of water in contact with the brush may lead to preferential adsorption of hydroxide and hydronium ions depending on the pH.

H.J. Kreuzer, R.L.C. Wang, and M. Grunze, New Journal of Physics 1, 21.1 (1999). R.L.C. Wang, H.J. Kreuzer, and M. Grunze, Phys. Chem. Chem. Phys. 2, 3613 (2000). L. Livadaru, H.J. Kreuzer, and R.R. Netz. Interacting Chain Model for Poly(ethylene glycol) from First Principles. Macromolecules (in press). Kreuzer, H.J.; Payne, S.H.; Livadaru, L. Biophysical Journal 2001, 80(6), 2505-2514. Kreuzer, H.J.; Grunze, M. Europhys. Lett. 2001, 55(5), 640-646. M. Himmelhaus, T. Bastuck, S. Tokuitsu, M. Grunze, L. Livadaru and H.J. Kreuzer. Growth of a polymer brush from solution, (preprint).

10:20am **BI-MoM7 Puzzles of Fluid Flow in the Biomaterials Environment**, ***S. Granick***, University of Illinois, Urbana-Champaign **INVITED**

In areas from blood flow to biosensor applications, it is essential to predict fluid flow. The standard model states that fluid velocity is zero at solid surfaces, but evidence is accumulating against this in many situations, especially regarding aqueous solutions and surfaces coated with polymer cilia. We have studied flow of aqueous solutions containing variable amounts of monovalent and divalent electrolyte past solid surfaces whose charge was varied and whose 'softness' was varied by polymer cilia. Deviations from the standard model are observed when the wall shear stress exceeds a critical level whose magnitude depends on the system studied. In some respects this is understood, in other respects it is not. The puzzles will be emphasized.

11:00am **BI-MoM9 Hydration Forces on a Switchable Bioactive Surface**, ***B.-I. Kim***, *M.A. Samara, D.L. Huber, J.E. Houston, B.C. Bunker*, Sandia National Laboratories

Poly(n-isopropyl acrylamide) (PNIPAM) monolayers can be thermally switched between hydrophobic and hydrophilic states at a phase transition temperature of 35°C. Protein adsorption studies indicate that the hydrophilic state represents an anti-fouling state, while biomolecules form adherent monolayers on the hydrophobic state. We have used a scanning probe system called the interfacial force microscope (IFM) to probe the mechanisms for protein adsorption on this switchable polymer surface. With the IFM, we have simultaneously measured both normal and friction forces between a silica tip and a surface functionalized with PNIPAM as a function of separation distance. The results show that at room temperature, there is a repulsive hydration force between the tip and the substrate. As the phase transition temperature is approached, the repulsive force collapses, allowing the tip and substrate to come into adhesive contact. The transition from repulsive to attractive adhesive forces is accompanied by a doubling in lateral friction forces. IFM results obtained at different tip speeds at different temperatures suggest that the repulsive hydration force observed at room temperature is associated with the presence of ordered water structures within the polymer that break down at higher temperatures. Experiments are in progress with chemically-functionalized tips to provide us with fundamental insights of the parameters controlling the stability of this ordered water and its role in protein adsorption.

11:20am **BI-MoM10 Structural Properties of Nucleosomal DNA Characterized by Atomic Force Microscopy**, ***M.E. Greene***, *M.A. Ratner, J. Widom, M.C. Hersam*, Northwestern University

One of the fundamental problems in contemporary molecular biology involves whether a sequence dependence exists in nucleosomal DNA which gives the molecule certain structural properties leading to the formation of nucleosome with histone octamer. A way to approach the solution is to look at the isolated DNA molecules to discern the native structural properties in the absence of histones. Interfacing biological molecules with inorganic substrates and probing them using atomic force microscopy (AFM) allows for such study. AFM has been used to image surfaces with adsorbed biological molecules for over a decade, and in particular DNA has been characterized to an extent that imaging artifacts interfering with proper analysis have been identified. Several technical difficulties have been resolved as well, including substrate selection and a reproducible surface binding protocol, opening the door for AFM to be used as a powerful tool to investigate problems of genuine biological importance. In this investigation, a 342-bp strand of synthetic dsDNA dubbed "601" shown by Lowary and Widom to have a high affinity for binding to histone octamer is examined. This sequence is thought to mimic the behavior of DNA sequences found in chromatin. Preliminary analysis of AFM data of a natual nucleosomal DNA sequence isolated from chicken erythrocyte suggests agreement with the

worm-like chain (WLC) model. Attention is given to the quantities of end-to-end distance, contour length, and intrachain bend angles in order to assess the persistence length, bendedness, and bendability of the sequence. AFM data is currently being gathered using Si cantilevers tipped with multiwalled carbon nanotubes as well as high aspect ratio Si tips with a nominal radius of curvature of 2 nm to obtain better lateral resolution and detailed measurements of bends and curvature fluctuations in the chains. An automated analysis methodology to allow the handling of large data sets will be introduced as well.

Biomaterials
Room: C-201 - Session BI+VT-MoA

Protein Surface Interactions
Moderator: D. Grainger, Colorado State University

2:00pm **BI+VT-MoA1 Self-Assembled Monolayers of Carboxy-Terminated Poly(ethylene glycol): Protein Resistance, Biospecific Functionalization and Application to Immunodiagnostics**, ***R. Dahint***, University of Heidelberg, Germany **INVITED**

The high specifity of antigen/antibody reactions has been widely exploited to develop accurate detection methods for biomolecules. Heterogeneous immunoassays, where proteins are selectively bound by immobilized antibodies and detected by the use of labeled secondary antibodies are a standard diagnostic technique. Also, a considerable amount of research has been focused on immunosensor development. A general problem in immunodiagnostics is non-specific protein adsorption: Macromolecules are not only bound to the substrate by specific antigen/antibody recognition, but also adhere due to non-specific interaction forces. Hereby, the accurate determination of antigen concentration may be significantly deteriorated. Moreover, non-specifically adsorbed proteins may even block and deactivate the immobilized receptors. The integration of specific receptors into a protein resistant matrix would, therefore, significantly improve quantitative analysis. Self-assembled monolayers (SAMs) of poly- and oligo(ethylene glycol) have proven to effectively prevent protein adsorption. We, therefore, synthesized a carboxy-terminated poly(ethylene glycol) alkanethiol ($HOOC\text{-}CH_2\text{-}(OCH_2\text{-}CH_2)_n\text{-}O\text{-}(CH_2)_{11}\text{-}SH$, n = 22-45) which facilitates covalent coupling of antibodies. In contrast to most other previous studies, where receptors have been coupled to SAMs formed from a binary mixture of differently functionalized molecules, only a single chemical functionality is involved. After characterizing the films by infrared absorption (FTIR) and X-ray photoelectron spectroscopy, ellipsometry and contact angle measurements, their performance as bioselective coatings with reduced non-specific adsorption has been tested in both FTIR and acoustic wave sensor experiments. The protein resistant properties of the films are put in context with previous results on oligo(ethylene glycol) alkanethiolate SAMs including neutron reflectivity studies on protein/surface interactions.

2:40pm **BI+VT-MoA3 ToF-SIMS and XPS Analysis of Enzymatic Digests of Adsorbed Protein Films**, ***M.S. Wagner***, *D.G. Castner*, University of Washington

Characterization of multicomponent adsorbed protein films is critical in understanding biological interactions with surfaces. We have previously shown that Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) can quantify the composition of binary and ternary adsorbed protein films using the low mass ($0 < m/z < 200$) fragmentation pattern of the mass spectrum. However, quantification of more complex protein films using this method is limited to the most abundant proteins present in the films. Therefore, trypsin digestion of the adsorbed proteins was performed to generate tryptic fragments for subsequent analysis by ToF-SIMS. The tryptic fragments were identified by combining ToF-SIMS with matrices from Matrix Assisted Laser Desorption and Ionization Mass Spectrometry (MALDI-MS). Residual protein remaining on the substrate after digestion was detected using ToF-SIMS and X-ray Photoelectron Spectroscopy (XPS). This method provides unique fragments for the identification of adsorbed proteins by ToF-SIMS.

3:00pm **BI+VT-MoA4 Protein Adsorption on Colloidal Oxide Particles**, ***K. Rezwan***, *L.P. Meier, M. Textor, L.J. Gauckler*, ETH Zurich, Switzerland

Protein adsorption to surfaces of medical implants is an essential aspect of the cascade of biological reactions taking place at the interface between synthetic material and biological environment. The types and amounts of adsorbed proteins mediate subsequent adhesion, proliferation and differentiation of cells as well as deposition of mineral phases. Most metallic biomaterials are covered by a protective, stable oxide film such as titanium oxide on titanium. Hence proteins only interact with the oxide film and not with the underlying metal. Closer investigations of the protein - oxide interface are therefore of great relevance to the biomaterials field. In the past, protein adsorption and desorption has been investigated mostly on planar surfaces by in situ techniques such as ellipsometry, optical waveguide lightmode spectroscopy (OWLS) and quartz crystal microbalance (QCM). The drawback of these methods is the lack of direct information about surface charges, which are known to strongly affect protein adhesion and conformation at interfaces. We used colloid chemistry analysis methods such as colloidal vibration potential (CVP), X - ray disc centrifuge (XDC) measurements and UV â€“ spectroscopy (UVS) to study in detail the adsorption of proteins to well-defined colloidal particles of typically 100 - 200 nm diameter. Combining these methods, the adsorbed amount of proteins and its influence on the zetapotential and the isoelectric point of the particles were determined with great precision and across a wide pH range. Adsorption of bovine serum albumin was found to alter the zetapotential of the oxides Al2O3, TiO2 and SiO2 and their isoelectric points to an extent that depended on the adsorbed mass. Combining UVS and XDC, the volume density and the thickness of the protein layer could be determined. The thickness corresponded to a monolayer or less. The adsorbed mass of albumin turned out to be nearly independent of pH in the range from pH 2 to 12.

3:20pm **BI+VT-MoA5 Prediction of Adsorption Behavior of Fibronectin as a Function of Surface Functionality Using a Customized Protein Adsorption Force-Field**, ***R.A. Latour***, *K.A. Wilson*, Clemson University, *A.J. Garcia*, Georgia Institute of Technology, *S.J. Stuart*, Clemson University

The ability of a cell to bind to an adsorbed protein layer on a biomaterial surface is dependent on the structure and availability of the protein's cell binding domains following adsorption. A well-known example of this is integrin binding to the PHSRN and RGD sites located on the 9th & 10th type III repeats of fibronectin (Fn). The objective of this research was to utilize computational chemistry to predict the relative orientation and accessibility of these cell-binding domains in Fn after adsorption as a function of surface functionality (CH3, OH, NH3+, COO-). Modeling was conducted using an SGI O2/Onyx computational system with InsightII software (Accelrys). The Charmm force-field was used to simulate intramolecular interactions for the fibronectin, while a new set of force-field parameters was created to simulate the interactions between the fibronectin and the surface. The new force-field parameters were set to provide similar energy vs. surface separation plots for peptide residue-surface adsorption as determined by previous semi-empirical modeling studies using MOPAC/PM3/COSMO. Initial energy vs Fn orientation maps were generated followed by 50 ps molecular dynamics simulations at selected positions to assess initial adsorbed Fn behavior. Results suggest that the CH3 and COO- surfaces should most strongly inhibit integrin binding, but by different mechanisms; the CH3 surface by disrupting Fn structure and the COO- surface by blocking accessibility. The OH and NH3 surfaces were predicted to preserve binding site structure and accessibility. Results compare favorably with experimental studies and provide likely molecular mechanisms that help explain experimentally observed behavior.

3:40pm **BI+VT-MoA6 Analysis of Organic and Biological Materials in Ultra-High Vacuum**, ***D.G. Castner***, University of Washington **INVITED**

Ultra-high vacuum (UHV) surface science has a long, successful history in the fields of catalysis and microelectronics. The early adaptation of UHV-based tools in these fields was largely due to the fact that the materials involved (metals, ceramics, semiconductors, etc.) were readily vacuum compatible. This talk will address the challenges of adapting UHV surface analysis techniques for analyzing organic and biological materials. These include their higher vapor pressure, their increased susceptibility to X-ray, electron, and ion sample degradation, and vacuum induced changes in their structure. Some of the first organic surface analysis experiments were done on polymers. Since that time, these experiments have been extended to self-assembled monolayers (SAMs), biomaterials, and adsorbed biomolecules. Examples to be discussed from these areas will include the effect of polymer additives, surface rearrangement of polymers, the well-defined structure of SAMs, and preserving the conformation of adsorbed proteins.

4:20pm **BI+VT-MoA8 PEG-ylated Surfaces with Graded Protein Interactiveness : A ToF-SIMS, XPS and Optical Waveguide Sensor Study**, ***S. Pasche***, *S.M. De Paul, J. Vörös*, Swiss Federal Institute of Technology, *P. Hug, B. Keller*, Swiss Federal Laboratory for Material Testing and Research, *H.J. Griesser*, University of South Australia, *N.D. Spencer, M. Textor*, Swiss Federal Institute of Technology

Poly(L-lysine) grafted with poly(ethylene glycol) (PLL-g-PEG), a polycationic co-polymer positively charged at neutral pH, has been shown to spontaneously adsorb onto negatively charged surfaces, rendering them protein-resistant to a degree related to the PEG surface density. Since the PEG surface density is a function of polymer architecture (PEG molecular weight and grafting ratio expressed as number of lysine monomers per PEG side chain), it becomes feasible to control the interactiveness of a surface by varying the co-polymer architecture. Angle-dependent XPS and ToF-SIMS

were used to investigate the surface-chemical properties. The adsorbed mass after serum exposure was determined by an optical sensor technique. Further colloid-modified AFM force measurements aim at studying the mechanical properties of the coated surfaces. PLL-g-PEG was adsorbed onto niobium oxide coated wafers, resulting in the formation of stable polymeric monolayers. The grafting ratio, g, of the polymer was varied systematically between 2 and 10, leading, upon surface adsorption, to highly different, but controlled PEG surface densities. PEG molecular weight was varied between 1000 and 5000. Polymer adsorbed mass was determined quantitatively by an in situ optical waveguide technique. A quantitative relationship was established between EG-monomer surface density, calculated from the known polymer architecture and the surface-adsorbed mass, ToF-SIMS intensities of PEG-, PLL- and substrate-related secondary ion peaks, and the amount of serum proteins that adsorbed onto the different polymer-coated surfaces. PLL-g-PEG surface-coating technology allows the fabrication of surfaces with tailored interactiveness and the establishment of design criteria for PEG-based, protein-resistant surfaces.

4:40pm **BI+VT-MoA9 Time-of-Flight Secondary Ion Mass Spectrometry Analysis of Conformational Changes in Adsorbed Protein Films**, ***N. Xia***, University of Washington, *C.J. May*, Yale University, *S.L. McArthur, D.G. Castner*, University of Washington

Characterizing the identity, composition, conformation, and orientation of adsorbed proteins is essential for the development of biocompatible devices. Static time-of-flight secondary mass spectrometry (ToF-SIMS) is a powerful surface analytical technique for analyzing adsorbed protein films. However, the ToF-SIMS experiment is done under vacuum, and drying adsorbed proteins for analysis can denature or change their conformation. In this study, trehalose coating was used to inhibit these conformational changes from occurring during sample preparation for ToF-SIMS analysis. Surface plasmon resonance (SPR) analysis showed that air-dried films of trehalose-stabilized antibodies retained a significant proportion of their hydrated antigen binding activity. In contrast, air-drying without trehalose protection resulted in the adsorbed protein films losing most of their antigen binding activity. Structural differences between trehalose-stabilized and unstabilized protein films were then analyzed with static ToF-SIMS. By application of principle component analysis (PCA) to the ToF-SIMS spectra, the biological activity difference observed in SPR was correlated to changes in protein conformation. Trehalose-protected proteins retained a greater degree of their original conformation than the unprotected proteins. This suggests that static ToF-SIMS has the capability to distinguish conformational differences in adsorbed protein films. Moreover, trehalose protection can be used for static ToF-SIMS analysis of adsorbed protein films to obtain structural information that is more relevant to the structure of the proteins in aqueous conditions.

5:00pm **BI+VT-MoA10 Study of the Adsorption Kinetics and Conformational Changes of Human Serum Albumin and Human Plasma Fibronectin using PM-RAIRS, Radiolabelling and Atomic Force Microscopy**, ***R.J. Manning***, *C.M.J. Fauroux, M.J. Pilling, P. Gardner, G.J. Leggett*, University of Manchester Institute of Science and Technology, UK

The kinetics of adsorption of proteins has been studied on self assembled monolayers (SAMs) on gold, formed by the adsorption of alkanethiolates with differing functional groups and varying alkyl chain lengths. The adsorption of human serum albumin (HSA) and human plasma fibronectin (HPF) has been studied using three complementary techniques: post modulation fourier transform reflection adsorption infrared spectroscopy (PM-RAIRS), radiolabelling, and atomic force microscopy (AFM). Initial adsorption kinetics of HSA and HPF were established using FTIR. It was found that monolayer coverage was reached faster on methyl terminated SAMs than on hydroxyl and carboxylic acid terminated hydrophilic monolayers. Tritium radiolabelling of HSA and HPF confirmed the trends observed with FTIR. The conformations of the adsorbed proteins were followed using PM-RAIRS, enabling quantitative monitoring of the percentage of α-helix, β-sheet, β-turn and random coils, indicating the degree of denaturation on differing surfaces over time. Finally, AFM was used to generate direct observations of layers of adsorbed proteins, providing useful insights into the distribution of proteins across the differing surfaces and enabling individual molecules to be observed. HSA was found to form a fibrillar network on methyl terminated SAMs at low concentrations and short adsorption times, whilst individual molecules were observed on hydroxyl and carboxylic acid terminated monolayers. This study demonstrates the complimentarity of FTIR, radiolabelling and AFM in understanding the adsorption of proteins on well-ordered SAMs.

Nanometer Structures
Room: C-207 - Session NS+BI-MoA

Nanobiology
Moderator: V. Vogel, University of Washington

2:20pm **NS+BI-MoA2 Molecular Shuttles Based on Motor Proteins: Transporters for Nanotechnology**, ***H. Hess***, *J. Clemmens*, University of Washington, *C.M. Matzke, G.D. Bachand, B.C. Bunker*, Sandia National Laboratories, *J. Howard*, Max-Planck-Institute of Molecular Cell Biology and Genetics, Germany, *V. Vogel*, University of Washington

Active transport in cells, utilizing molecular motors like kinesin and myosin, provides the inspiration for the integration of active transport into synthetic devices. Hybrid devices, employing motor proteins in a synthetic environment, are the first prototypes of molecular shuttles - an active nanoscale transport system. The key problems for the construction of a molecular shuttle are guiding the direction of the motion, controlling the speed, and loading and unloading of cargo. Various techniques, relying on surface topography and chemistry as well as flow fields and electric fields, have been developed by us[1] and others[2] to guide the movement of molecular shuttles on surfaces. The control of ATP concentration, acting as fuel supply, can serve as a means to control the speed of movement. The loading process requires the coupling of cargo to the shuttle, ideally by a strong and specific link. Applications of molecular shuttles can be envisioned e.g. in the field of Nano-Electro-Mechanical-Systems (NEMS), where scaling laws favor active transport over fluid flow, and in the bottom-up assembly of novel materials. Recently, we demonstrated that the shuttles can be employed as self-propelled nanoscale probes to image surface topography.[3] The construction of an image relies on the tracking of the random movement of a large number of independent probes, a concept which is fundamentally different from e.g. the deterministic movement of a single tip in scanning probe microscopy. An aspect of our research is that devices using molecular shuttles can be based on mechanisms which are unique to the microscopic world. An example of this is the possible application of a Brownian ratchet for directional sorting.[4]

[1] Hess, H., et al., Nano Letters, 2001. 1(5): p. 235.
[2] Hess, H. and Vogel, V., Rev. Mol. Biotechn., 2001. 82: p. 67.
[3] Hess, H., et al, Nano Letters, 2002. 2(2): p. 113.
[4] Hess, H., et al., Appl. Phys. A, 2002. 75: p. 309.

2:40pm **NS+BI-MoA3 Nanomechanics of an Intrinsically Unstructured Protein**, *R. Mukhopadhyay*, ***J.H. Hoh***, Johns Hopkins School of Medicine **INVITED**

Microtubule-associated proteins (MAPs) are a class of proteins that bind to the surface of microtubules. These proteins are known to stabilize microtubules against depolymerization, and there evidence to suggest that MAPs play a role in maintaining spacing between adjacent microtubules and may play a role in cellular mechanics. MAPs are composed of two domains: the microtubule binding domain and the projection domains. Biophysical studies of the projection domain suggest that it is highly unstructured. We have recently developed a system to study the molecular mechanics of the projection domain of MAPs. In this system MAPs are end-grafted by their positively charged microtubule binding domains to a negatively charged surface. The properties of the projection domain are then probed by direct atomic force microscope (AFM) force measurements. These measurements show a long-range repulsive force that extends more than 100 nm from the surface, and is consistent with a polymer brush like interaction. A unique thrombin cleavage site at the boundary between the microtubule binding domain and the projection domain allows the projection domain to be proteolytically removed. This results in a total loss of the long-range repulsive force. The force is also sensitive to ionic strength, suggesting that, consistent with its sequence, the projection domain behaves as a polyelectrolyte. The polyelectrolyte nature of the projection domain and the large number of phosphorylation sites suggests a mechanism for regulating the mechanical properties of the protein. This notion is supported force measurements on phosphorylated and dephosphorylated MAPs. Thus phosphorylation of the MAP projection domain offers a biochemical mechanism for modulating the molecular mechanics of MAPS and the intermolecular forces between microtubules.

3:20pm **NS+BI-MoA5 Nanostructures for Analysis of individual Biomolecules**, ***H.G. Craighead***, *S.W. Turner, M. Foquet, J. Korlach, W. Zipfel, M. Levene, W.W. Webb*, Cornell University

We have used nanofabrication methods to create fine-scale fluid channels and optical devices for nano-scale spatial confinement of optical excitation for use in the analysis of individual biomolecules. Functional fluid systems with dimensions down to ~35 nm have been etched and created by use of sacrificial layer techniques. Narrow fluid channels have been used for DNA

fragment sizing by single molecule analysis and used for fluorescence correlation spectroscopy with improved signal-to-noise ratios. Related lithographic approaches have been used to create regions of optical excitation, confined in all 3 dimensions, using metallic nano-constrictions or "zero mode waveguides" in which electromagnetic waves are exponentially attenuated. These devices enable practical studies of dynamic biochemical processes at the single molecule level. An example of such a process is the observation of the activity of a single DNA polymerase molecule during the replication of a DNA molecule. We have been able to optically observe the incorporation of individual bases in the DNA replication process. With optically differentiated base types, this could lead to high speed sequencing of single DNA molecules. These approaches may allow highly parallel observation and analysis of biochemical activity at the single molecule level. This work has been supported by The National Institutes of Health, the National Science Foundation through the NBTC and the Department of Energy. Fabrication of devices was done at the Cornell Nanofabrication Facility.

3:40pm **NS+BI-MoA6 Polyelectrolyte Multilayer Self Assembled Nanoparticles for Delivery of Transforming Growth Factor Beta**, ***C. Catuogno***, *M. Tabrizian*, McGill University, Canada

Biocompatible and biodegradable nanoparticles with additional high drug encapsulation efficiency and controllable targeting would form an ideal drug delivery system. Investigation of the possibility of making such vehicles is presented in this work. Such devices could be used in combination with polymeric bone scaffolds for delivery of transforming growth factor beta (TGF-b) in order to accelerate bone cell differentiation and bone formation in vitro. The particle shell is made of natural carbohydrate polymers namely chitosan or hyaluronic acid that are commonly used in tissue engineering. Chitosan is insoluble in water or in alkaline solutions but dissolves readily in dilute solutions of most organic acids. Chitosan has gel-forming ability at low pH and swells in acidic medium or in water. These proprieties added to those of hyaluronic acid have motivated the use of both materials to control TGF-b adsorption and release from the nanoparticles. Additionally, composite materials have been shown to improve mechanical properties of scaffolds. The nanoparticles are built from a succession of polyelectrolytes using the layer-by-layer method. Colloidal iron oxide particles are adsorbed on the polymer layers and encapsulated into the nanoparticles. This aims at inducing the guidance of nanoparticles using an external magnetic field to desired site of delivery when such device is used for in situ bone generation.

4:00pm **NS+BI-MoA7 Resolving Scanning Tunneling Microscopy Features of Oligomers Adsorbed on Si(100)**, ***B. Grandidier***, *Mathieu Dubois, C. Delerue, J.P. Nys, D. Stievenard*, IEMN, France, *J. Roncali*, IMMO, France

Scanning tunneling microscopy (STM) gives the possibility to observe complex organic molecules on silicon surfaces in ultra-high vacuum. Although the reactivity of the silicon surfaces often leads to the modification of the molecular electronic states, a clever synthesis of the molecules can keep their structures intact after adsorption. As a result, the molecules are imaged in their integrity and the contrast variation observed along the molecules is usually associated with the highest occupied and lowest unoccupied electronic states of the molecules. Here, we have investigated the STM imaging of conjugated oligomers on Si(100) by tight binding simulations. The molecules are physisorbed on the surface and extend over a few dimers rows. Due to coupling of the molecular states with the electronic states of the silicon surfaces, we show that the off-resonance tunneling process is the major contribution to the tunneling current in usual tunneling conditions. As a result, the potential barrier is lowered when the tip scans above a molecule and the contrast variation gives an enhancement of the Si dimer rows. Experimental STM images of conjugated oligomers confirm this theoretical prediction.

4:20pm **NS+BI-MoA8 Q-dots Patterned Surfaces for Cell Adhesion**, *A. Szucs*, ***J.P. Spatz***, University of Heidelberg, Germany

Highly luminescent semiconductor quantum nanodots (Q-dots) regularly patterned on different substrates, were synthesized and applied as binding sites for single cell receptors in order to study cell adhesion. CdS, CdSe and CdTe/ Q-dots in the size range of 2-8 nm in diameter were generated in Poly (styrene-b-2-vinyl-pyridine) (PS-P2VP)/inorganic hybrid reverse micellar system (RM). Solid Cd salts loaded polymer cores, constructed by the 2-vinyl-pyridine, were used as nanocompartments for preparation of uniform semiconductor nanoparticles. Particle size could be controlled by varying the diameter of the RM core, via the length of the core constructing polymer, and by changing the precursor salt loading in the polymer core. Different kinetics and structures were observed inside the RM core during the particle formation by using different precursor salts (Cd(ClO4)2, Cd(OAc)2). Photo luminescent properties of semiconductor nanoparticles were investigated by different methods (UV-VIS spectrophotometry, steady-state fluorescence, color luminescence imaging). "In situ" surface patterning on different substrates (Glass, Si-wafer etc.) by self-organization of the diblock copolymer micelles on the surfaces was monitored by AFM measurements.

4:40pm **NS+BI-MoA9 Cell Adhesion to Nanostructured Interfaces**, ***J.P. Spatz***, University of Heidelberg, Germany **INVITED**

Nanostructures with micrometer or nanometer spacings have been prepared through pure self-assembly of diblock copolymer micelles (formation and compartmental localization of metallic nanodots within block copolymer micelles) or in combination with a top-down approach (electron beam lithography). Within these structures, 7 nm Au particles can be positioned with a precision of < 10 nm and large freedom in pattern choice (periodic, aperiodic, dotes, lines). Specifically, this is obtained by casting a solution of HAuCl4 loaded block copolymer micelles onto the prestructured resist film. Due to capillary effects and steric hindering, the particles are centered within the prepatterned holes and at the edges of prestructured lines. Subsequent lift-off of the resist allows the removal of all micelles with the exception of those that are in direct contact to the underlying substrate. The block copolymer is then removed by plasma etching, which strips the polymer micelle and reduces the gold salt to gold thereby leaving behind nanoscopic dots or lines of gold in a defined array. These nanostructured interfaces are used as platform for biofunctionalisation of solid interfaces. The surfaces are used as a tool to investigate cluster formation of focal adhesion associated proteins of fibroblasts. Cultured human melanocytes allowed to study the regulation of cell shape through contact with interfaces offering different topography and biochemical pattern. The cellular morphology of melanocytes is a measurable indicator for cell reaction to the cellular environment. The characteristic cellular shape of different cell cultures was quantified by different shape parameters like the number and length of dendrites. A decreased signal-to-noise ratio was found for melanocyte cells concerning the number of dendrites and orientation of dendrites if cultured on biochemically and topographically structured substrates.

Biomaterials
Room: C-201 - Session BI+SS-TuM

Platforms for Non-fouling and Patterned Surfaces
Moderator: D.G. Castner, University of Washington

8:20am **BI+SS-TuM1 Molecular Assembly and Micro-/Nanopatterning Techniques on Oxide-based Surfaces for Controlling Non-specific and Specific Interactions**, ***M. Textor***, ETH Zürich, Switzerland **INVITED**

The assembly of multifunctional molecules at surfaces has become an important technique to design interfaces for biosensor applications and model surfaces for cell-biological studies. While alkanethiol self-assembled monolayers on gold surfaces are routinely used today, corresponding systems for oxide-based surfaces had first to be developed. The objective is to produce interfaces via cost-effective, robust techniques that allow the elimination of non-specific protein adsorption and the addition of ligands in controlled density to sense the biological environment. Poly(ethylene glycol)-grafted polyionic copolymers assemble spontaneously from aqueous solutions at charged interfaces resulting in well-defined, stable monolayers. The degree of interactiveness of the resulting surface with the bioenvironment can be controlled quantitatively through the design of the polymer architecture. If the polymer is functionalized with bioligands such as biotin, biosensor interfaces with quantitative control over ligand density can be efficiently produced. Chemical patterning of surfaces into adhesive and non-adhesive areas has become an important tool to organize in a controlled manner biological entities such as cells and biomolecules at interfaces. A novel surface modification technique is presented that uses a lithographically pre-patterned, inorganic substrate, which is subsequently converted into a pattern of biological contrast via area-selective molecular assembly processes. Biologically meaningful patterns of protein-adhesive and non-adhesive areas in a size range from micrometers to as small as 50 nm could be produced. Fluorescence microscopy, XPS, ToF-SIMS and AFM were used to control ex situ each surface modification step, while the kinetics of the surface reactions including the interaction with biological media were monitored in situ with an optical sensor (OWLS) and the quartz crystal microbalance (QCM-D) technique.

9:00am **BI+SS-TuM3 Orientation in Oligo(ethylene glycol) Functionalised Self Assembled Monolayers Adsorbed on Gold Depending on the Oligomer Length**, ***M. Zwahlen***, University of St Andrews, UK, *S. Herrwerth, W. Eck, M. Grunze*, University of Heidelberg, Germany, *G. Haehner*, University of St Andrews, UK

Oligo(ethylene glycol) (OEG) functionalised self-assembled monolayers (SAMs) have attracted considerable attention due to their protein repelling properties. The underlying mechanism is of high scientific relevance for future applications but has not yet been completely resolved. 'Steric repulsion', which describes the resistance to non-specific protein adsorption in the case of the polymer PEG does not explain the mechanism in densely packed SAM structures sufficiently. It has been suggested that one cru cial parameter for the interaction of OEG-modified surfaces with their environment is the orientation in the organic adlayer. This has motivated a number of structural investigations on OEG-SAMs. As a contribution to the ongoing discussion, we present a s tudy of the orientation in OEG-functionalised SAMs adsorbed on gold. It was measured as a function of the number of EG units in the molecule using soft X-ray absorption spectroscopy (NEXAFS). The results and their implications on the vacuum structure of the OEG-films will be discussed. The data will be compared to those obtained with complementary experimental techniques under similar as well as under different environmental conditions.

9:20am **BI+SS-TuM4 DOPA: A Novel Anchor for PEGylation of Biomaterials**, ***J.L. Dalsin***, *P.B. Messersmith*, Northwestern University

It is widely recognized that modification of biomaterial surfaces with biocompatible polymers is a useful strategy for controlling protein adsorption and cell interactions with materials. The physical or chemical immobilization of poly(ethylene glycol) (PEG) has routinely been used to limit biological fouling of surfaces. Many of the current PEGylation methods, however, are limited by high costs and complexity of synthesis. Most importantly, each of the present strategies vary widely depending on the characteristics of the substrate, and are typically different for metal, metal oxide, and polymer substrates. We are developing a new biomimetic strategy for anchoring PEG to biomaterial surfaces. Our approach is to utilize linear and branched PEGs end-functionalized with DOPA. DOPA is found in significant quantities in the adhesive proteins secreted by marine mussels for attachment to underwater surfaces, and recent evidence suggests that the presence of DOPA promotes strong and durable adhesion of these proteins to metal, metal oxide, and polymer surfaces. Recently, it has been shown that DOPA-containing peptides adhere strongly to gold surfaces, mediated by metal-oxygen bonds formed between the catechol group of DOPA and Au atoms at the metal surface. In this study, we report our findings on the use of DOPA as an anchor for PEGylation of biomaterial surfaces. A variety surfaces were modified by adsorption of DOPA-modified-PEGs from solution, and the presence of PEG on the surface was confirmed with a number of surface characterization techniques, including XPS and TOF-SIMS. The behavior of cells on modified and unmodified gold surfaces was evaluated in an attempt to optimize the conditions for DOPA-mediated PEGylation of metals, metal oxides, and polymers.

9:40am **BI+SS-TuM5 Characterization of Non-Fouling Surfaces by Matrix-Assisted Laser Desorption / Ionization Mass Spectrometry**, ***G.R. Kinsel***, *J. Zhang, R.B. Timmons, M. Li*, University of Texas at Arlington

Matrix-Assisted Laser Desorption / Ionization (MALDI) mass spectrometry has emerged in recent years as a powerful method for the mass spectrometric analysis of a wide range of biomolecules including proteins, oligonucleotides, polysaccharides, etc. An attractive feature of this analytical approach is the relative simplicity of the sample preparation. In principle, all that is required is that the analyte of interest be mixed with an appropriate "matrix" (typically a small, functionalized aromatic compound) and the two compounds allowed to co-crystallize on some type of support. In recent work, however, we have shown that the nature of the support can have a marked effect on the magnitude of the analyte MALDI ion signal. Specifically, we have shown that as the binding affinity of the support for the analyte increases, the analyte MALDI ion signal decreases. This relationship has been used to develop a quantitative method for the determination of the protein binding affinity of various materials based on a MALDI standard additions approach. In the present studies the MALDI method has been used to quantitate the protein binding affinity of a number of "non-fouling" surfaces. These surfaces include plasma polymerized PEO, plasma polymerized CH3OH, PEO-PU block copolymers, and PEO grafted surfaces. The "non-fouling" properties of these surfaces are compared with the protein binding affinity of other conventional polymers including PTFE, LDPE, etc. In addition, the binding properties of the various surfaces are examined with relation to a variety of peptides and modest sized proteins.

10:00am **BI+SS-TuM6 Polymerized Planar Biomembrane Assemblies**, ***S. Saavedra***, University of Arizona

The utility of planar supported lipid bilayers (PSLBs) as protein-resistant coatings in molecular device technologies is hampered by the chemical and mechanical instability of these structures relative to (for example) alkylsiloxane self-assembled monolayers. We have been investigating cross-linking polymerization of diene-functionalized lipids as a strategy to enhance the inherent instability of PSLBs. The membranes are self-assembled by vesicle fusion, then polymerized in situ by a redox-initiated chemistry. In contrast to diacetylene-based materials, these new diene-based materials contain relatively few defects. They are stable to conditions that would destroy a fluid membrane (e.g. exposure to air, surfactants, solvents), yet retain the characteristic protein resistance of a fluid PSLB. Thus these structures appear to possess both the stability and inertness required for implementation of PSLBs in many technological applications. This talk will focus on preparation, characterization, and protein functionalization of diene-based PSLBs.

10:20am **BI+SS-TuM7 Protein Binding at Biomembrane Interfaces**, ***P.S. Cremer***, Texas A&M University **INVITED**

We have used a combination of lithographic patterning techniques and microfluidics to spatially address fluid phospholipid bilayers at the liquid/solid interface. These systems are capable of multivalent ligand-receptor attachment chemistry. Moreover, on-chip designs allow for high throughput temperature, concentration, pH, and ionic strength measurements in an environment which closely mimics a cell membrane interface.

11:00am **BI+SS-TuM9 Surface Characterisation of Supported Lipid Layers**, ***S.L. McArthur***, *M.W. Halter, V. Vogel, D.G. Castner*, University of Washington

The boundaries of biological cells and organelles are defined by complex and dynamic membranes constructed from an array of lipids, proteins and carbohydrates. These interfaces have a range of specific functions and properties, one of which is their ability to prevent non-specific protein adsorption, making membrane mimics an attractive option for a variety of in vivo and in vitro biomedical implant and diagnostic applications. The

development and characterization of complex biomimetic surfaces presents a challenge in terms of their initial formation, long-term stability and integrity in a variety of environments and the maintenance of bilayer fluidity. In this study we detail the development and chemical characterization of supported lipid monolayers. The structure was formed by coupling HEMA to a glass support and subsequently activating it with CDI to couple the headgroups of the lipid, dimyristoyl ethanolamine (DMPE). The success of the immobilization procedure was investigated by XPS and ToF-SIMS. A number of different lipid transfer regimes were explored. Results illustrated that the samples produced using Langmuir-Blodgett transfer at high pressure (20 mN/m) had the largest fraction of the transferred lipids remaining at the surface after 5 minutes sonication in ethanol. Fluorescence microscopy of the lipid layers showed that the presence of this limited number of anchored lipids acted to stabilize the monolayer and maintain its integrity without having a detrimental effect on layer fluidity.

11:20am **BI+SS-TuM10 Purification of Mobile, Membrane-tethered Proteins in Micropatterned Supported Lipid Bilayers**, ***L. Kam***, *T.D. Perez, W.J. Nelson, S.G. Boxer*, Stanford University

Supported lipid bilayers are a unique system for studying fluidic membranes in a controllable in vitro format. A variety of methods for tethering proteins to supported bilayers provide a powerful reductionist model of cell-cell recognition and activation. However, contemporary methods for preparing membrane-tethered protein systems typically incur an immobile fraction; this population complicates and, at worst, subverts interpretation of experimental results. Here, we present a method for separating a population of mobile, GPI-tethered protein from an immobile fraction in a supported lipid bilayer. A GPI-modified protein based on the cell-cell adhesion protein E-cadherin was introduced into Egg PC vesicles by detergent dialysis. On a glass substrate, two adjacent and connected regions of supported lipid bilayer were created using a converging flow configuration. One region contained both mobile and immobile populations of GPI/cadherin while the other contained Egg PC alone. An electric field applied tangentially to the surface induced migration of the mobile, but not immobile, protein into the region of Egg PC, generating a purified population of these proteins which may then be isolated for analysis or further experimentation free from the immobile fraction. Importantly, this method is independent of the specific factors influencing protein mobility and thus generally applicable.

11:40am **BI+SS-TuM11 Spatial Control of Cell Attachment Using Micropatterned Plasma Polymers**, ***S.A. Mitchell***, *N. Emmison*, The Robert Gordon University, Scotland, UK, *A.G. Shard*, Sheffield University, England, UK

In recent years, there has been increased interest in the spatial control and regulation of cellular attachment and growth. Several techniques have been developed to produce surfaces with a well-defined chemical heterogeneity that are suitable for the rapid adhesion, spreading and proliferation of cells. Spatial control and sub-cellular pattern resolution has been successfully demonstrated by techniques such as micro-contact printing of self-assembled monolayers.[1] However, the labour intensive, time consuming preparation and ready oxidation of these surfaces limit the utility of these devices. Additionally, they are only applicable to substrates that are rarely used in biomedical devices. We have employed plasma polymerisation as an alternative method for the chemical patterning of surfaces, although the chemical composition of these surfaces is more difficult to control, this one-step procedure is rapid and cost effective.[2] The resulting surfaces have both a chemical functionality and a pattern resolution comparable to alternative techniques.[3] They may be applied to virtually any substrate, including relatively rough surfaces such as tissue culture polystyrene, greatly increasing their applicability. We describe the patterned deposition of plasma polymers onto a variety of substrates and outline some of the advantages and limitations of the technique. Physicochemical characterisation of the plasma polymers is performed with XPS, AFM and contact angle analysis. The culture of mammalian cells on patterned substrates demonstrates their ability to spatially regulate cell attachment and spreading.

[1]M Mrksich and G M Whitesides, Tibtech, 13, 228-235(1995)

[2]N A Bullett, R D Short, T OLeary, A J Beck, C W I Douglas, M Cambray-Deakin, I W Fletcher, A Roberts, C Blomfield, Surf. Interface Anal., 31, 1074-1076(2001)

[3]L Dai, H J Griesser, A W H Mau, J. Phys. Chem. B, 101, 9548-9554(1997).

Tuesday Afternoon, November 5, 2002

Biomaterials
Room: C-201 - Session BI+SS-TuA

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

2:00pm **BI+SS-TuA1 Medard W. Welch Award Address: The Biointerface Examined in Five Dimensions**, ***B.D. Ratner****, University of Washington **INVITED**

Twenty years ago, we had no such word as "biointerface." Now we use that word almost routinely to suggest surface-localized events between biological systems and solid surfaces (solids that vary in solidity from almost fluid to hard). To examine with a new perspective this burgeoning field, five facets of the biointerface will be explored. The five faces of the biointerface will be: temporally (and historically), spatially, molecularly, entrepreneurially and virtually (in computer space). Studies from our groups at the University of Washington and from others in the field will be presented. The talk will aim at defining the new field of the biointerface, relating it to "classical" surface science and highlighting opportunities.

2:40pm **BI+SS-TuA3 "Smart" Biomolecular Conjugates**, ***P.S. Stayton****, A.S. Hoffman, N. Malmstadt, C. Hu, S. Kulkarni*, University of Washington

One of the hallmarks of biological systems is their ability to change important properties in response to environmental cues. We have been developing stimuli-responsive biomolecular materials for biosensors, diagnostics, affinity separations, microfluidic devices, and chip/array devices that exhibit responsiveness to specific environmental cues. For many of the diagnostic and sensor technologies that utilize biomolecular recognition properties, there is a continuing need for better control routes. Current environmental methods are relatively harsh and can lead to damage of biomolecules and cells. In addition, the environmental signals are typically large general solution changes and thus not targeted to selective recognition components. The stimuli-respon sive biomolecular materials allow reversible control over protein recognition properties by utilizing small changes in environmental conditions or signals. The "smart" polymers reversibly cycle between an extended and hydrophilic random coil, and a collap sed, hydrophobic state that is reduced in average volume by ca. 3-fold. When the smart polymers are attached at defined protein side-chains, typically by genetically engineering cysteine or lysine residues, the polymers serve as sensors and actuators to c ontrol access of ligands or su bstrates to binding or catalytic sites. This general approach targets mild environmental signals to specific polymer-protein conjugates, and thus for example allows differential control of different antibodies in a device by using conjugated polymers that are sensitive to different signals (e.g. antibody 1 with pH, antibody 2 with temperature, antibody 3 with light). They can thus allow multiplexing control in complex mixtures, and are thus relevant to a number of different diagnostic and sensor formats.

3:00pm **BI+SS-TuA4 Molecular Recognition Mediated Fabrication of Protein Nanostructures by Dip-Pen Lithography**, *J. Hyun, S.J. Ahn, W. Lee,* ***S. Zauscher****, A. Chilkoti*, Duke University

The spatially controlled immobilization of biomolecules on solid surfaces at the nanometer length-scale is driven by the possibility of fabricating new sensors and actuators that will enable detection and actuation at the single molecule level. This communication describes how dip-pen nanolithography (DPN) in combination with the high-affinity streptavidin-biotin, protein-ligand system provides a simple and versatile "bottom-up" approach to create nanoscale biomolecular architectures in a step-wise fashion. This method involves the fabrication of nanoscale features by patterning a self-assembled monolayer (SAM) of a COOH-terminated alkanethiol on a gold substrate by DPN, followed by covalent immobilization of a high-affinity small-molecule ligand (biotin) onto the nanopatterned SAM and subsequent molecular recognition of its protein binding partner (streptavidin) from solution. We fabricated streptavidin nanostructures with lateral feature sizes in the range of 10-400 nm by this method, and have shown that the streptavidin nanopatterns can be used as a template to pattern biotinylated molecules of interest from solution. Because the binding of the final, target molecule is mediated by a highly specific molecular recognition interaction that occurs solely in the patterned region against a non-fouling background, this approach should allow patterning a biomolecule of interest directly from complex mixtures such as cell lysate without purification, which is not possible with alternative DPN methods that involve physisorption or covalent conjugation.

3:20pm **BI+SS-TuA5 Threading DNA Through a Nanopore: Applications for Analyte Detection**, ***J.J. Kasianowicz****, S.E. Henrickson, B. Robertson,* National Institute of Standards and Technology, *H.H. Weetall,* EPA, *M. Misakian,* National Institute of Standards and Technology **INVITED**

We recently demonstrated that single-stranded DNA (ssDNA) can be driven electrophoretically through a solitary Staphylococcus aureus alpha-hemolysin (alpha-HL) ion channel. In an effort to use this model system to understand DNA transport in biological systems, we show that the partitioning of ssDNA into the pore depends on the side to which the polymer is added and on the magnitude of the applied potential. Kramer's reaction rate theory was used to estimate both the height of the energy barrier for polymer translocation and the integral number of charges on ssDNA that interact with the barrier. In a related research effort, we illustrate three experimental results that suggest the interaction between polymers and a single nanopore can be used to quantitate analyte concentration and type. First, the probability that ssDNA enters the alpha-HL channel is proportional to the polymer concentration. Second, analyte binding to sites on ssDNA predictably alters the ability of the polymer to thread through the pore. Third, different ssDNA homopolymers induce current blockade patterns that are characteristic of the nucleotide type. We compare this method to other channel-based detection schemes. Finally, we show that modified polynucleotides might prove useful as "molecular rulers" for probing the structure of nanopores.

4:00pm **BI+SS-TuA7 Novel Immunosensor Interfaces based on Mixed Self-Assembled Monolayers of Thiols on Gold**, ***F. Frederix****, M. Boesmans, K. Bonroy, W. Laureyn, A. Campitelli,* IMEC, Belgium, *M.A. Abramov, W. Dehaen, G. Maes,* KULeuven, Belgium

The two components that make up a biosensor are the biological recognition layer, which selectively binds the analyte, and the transducer which translates this recognition event into an electrical signal. The increasing miniaturization of biosensor transducers (and thus of their active areas) and the demand for sensitivity, require a fully evaluated and optimized covalent immobilization of antibodies. Our research is therefore not only focusing on the transducer but also on the biological interface. This biological recognition layer mainly determines the specificity, stability, reproducibility, and durability of the biosensor as a whole. Our strategy is to achieve the above-mentioned properties based on mixed Self-Assembled Monolayers on gold. The realization of a biological recognition interface encompasses various aspects. Cleanliness and structural properties of the gold surface are very important for perfect SAM formation and were therefore optimized. Novel thiols able to couple antibodies or to mitigate non-specific adsorption were synthesized and evaluated, along with new molecules for blocking. The mixed monolayer formation of these novel thiols was characterized using contact angle measurements, XPS, cyclic voltammetry, and GA-FTIR. The immobilization of proteins on mixed SAMs is the most important step in the realization of immunosensors because it determines the activity of the antibodies and therefore the sensitivity. Random and orientated immobilizations of (chemically modified) antibodies on mixed monolayers of thiols were compared using Surface Plasmon Resonance. The enhanced sensitivity (< 0,1 ng/mL) and selectivity (no non-specific adsorption) were compared to commercially available biological recognition layers. In summary, we will show the importance of the biological recognition layer for the global performance of a biosensor and how the sensitivity can be drastically enhanced by modifications on the biological interface of an immunosensor.

4:20pm **BI+SS-TuA8 Electrostatic and Fluorescence Sensing of DNA Hybridization at Electrode Surfaces**, ***R.M. Georgiadis****, J. Wang, L.K. Wolf, A.W. Peterson,* Boston University

Current microarray technologies, based on specific probe-target hybridizations, often suffer from nonspecific surface interactions. In addition, for surface immobilized probes, thermodynamic equilibrium conditions may not be reached without excessively long incubation times and hybridization may be kinetically or sterically inaccessible for some probe sequences or for some surface probe densities. In previous work on perfectly matched duplexes, we have shown that probe density is a controlling factor for DNA hybridization at surfaces. Here, we expand our studies to investigate probe density effects for mismatched sequence or targets that access different binding locations on the immobilized probe. To improve mismatched hybrid discrimination we detect different dissociation

***** Medard W. Welch Award Winner

profiles for matched and mismatched 25-mer targets from surface-immobilized probes in the presence of an applied repulsive electrostatic field and present denaturation profiles for surface-bound hybrids obtained by continuously varying the applied electrostatic surface field. Finally, we examine the immobilization and hybridization of covalently-bound molecular beacons on gold surfaces using surface plasmon resonance (SPR) spectroscopy and fluorescence spectroscopy.

4:40pm **BI+SS-TuA9 Characterization of DNA on Gold: A Quantitative Surface Science Approach**, ***D.Y. Petrovykh***, University of Maryland - College Park / NRL, *H. Kimura-Suda, M.J. Tarlov*, National Institute of Standards and Technology, *L.J. Whitman*, Naval Research Laboratory

Covalent attachment of thiolated DNA onto gold surfaces is one of the most common methods for immobilizing aqueous DNA onto solid substrates. The formation of the DNA film in this case is thought to closely resemble that of alkanethiol self-assembled monolayers (SAMs). DNA films in modern applications, e.g. DNA microarrays, are < 10 nm thick with submonolayer surface coverage, which means that the traditional surface characterization techniques can be employed to complement biochemical analysis. We are applying XPS, FTIR, and ellipsometry to systematically quantify the chemical structure and coverage of self-assembled single-stranded DNA (ssDNA) films. Thymine (T) has the simplest structure of the four nucleotide bases: a single ring with two N atoms. Moreover, the environment of the two N atoms is so similar that the resulting XPS peak is consistent with a single N1s state in a polymer-like material. Backbone P atoms produce a base-independent single P2p peak. N and P are not subject to significant contamination, so the peak intensities can be used to estimate the coverage of immobilized DNA. N1s chemical shifts together with the base-dependent N/P ratio can confirm the presence of specific polynucleotides on the surface. The coverage determined by XPS is linearly correlated with base-specific IR features and agrees with absolute values obtained from radiolabeling measurements. We will also discuss how the XPS and IR spectra of dT-polynucleotide films can provide information about other basic properties of ssDNA films, such as uniformity and orientation, as well as issues of damage, degradation and contamination.

5:00pm **BI+SS-TuA10 Antibacterial Coatings of Immobilised Furanones**, ***H.J. Griesser***, *S. Al-Bataineh*, University of South Australia, *B.W. Muir, H. Thissen, M. Willcox*, CRC for Eye Research and Technology, Australia

The formation of bacterial biofilms and subsequent infections can cause serious complications in the use of biomedical devices such as catheters, and broadly effective technology is lacking. Nature has, however, addressed very effectively the problem of microbial colonisation of surfaces. For instance, the red alga Delisea pulchra secretes brominated furanones that prevent its microbial colonisation. These compounds are thought to interfere with bacterial quorum sensing by their chemical similarity with homoserine lactone, an important bacterial regulator. We have immobilised various furanones onto synthetic surfaces and tested the efficiency of such coatings in bacterial colonisation assays. A broadly applicable covalent immobilisation strategy involves nitrene chemistry, with light-activated reaction between furanones and azido aniline coupled onto a surface hydrogel interlayer. This allows coupling of furanones without reactive substituents but is non-selective with regard to molecular orientation and location of attachment. Other strategies require functionalised furanones, for example reaction between a hydroxylated furanones and surface isocyanate groups; such furanones can be more difficult to synthesize. Work to date has produced substantial reductions in bacterial colonisation, but not to the high degree required in clinical applications. Investigations now focus on the interactive effects of furanone molecular composition, immobilisation chemistry and surface density. An interesting finding is that these compounds are effective when surface immobilised although the classical microbiological model of homoserine lactone action requires entry into the microbial interior. This dichotomy calls for detailed surface characterisation of furanone coatings, including study of whether the entire population of surface-bound molecules is indeed covalently linked and thus non-diffusible and acting via a different mechanism to stifle bacterial colonisation.

Molecular and Bio-Magnetism
Room: C-205 - Session MB+BI+OF-TuA

Molecular and Bio-Magnetism
Moderator: M. Grunze, Heidelberg Universität, Germany

2:00pm **MB+BI+OF-TuA1 Single-Molecule Magnets: A Molecular Approach to Nanoscale Magnetic Materials**, ***G. Christou***, *M. Soler, N. Aliaga-Alcalde, S. Bhaduri*, University of Florida, *W. Wernsdorfer*, Laboratoire Louis Neel - CNRS, France, *D.N. Hendrickson*, University of California at San Diego **INVITED**

Single-molecule magnets (SMMs) are molecules that function as single-domain magnetic particles which, below their blocking temperature, exhibit the classical macroscale property of a magnet, namely magnetization hysteresis.[1] SMMs owe their properties to a combination of a large ground state spin value and easy-axis-type anisotropy, which give a significant barrier to magnetization relaxation. SMMs thus represent a molecular (or bottom up) approach to new nanoscale magnetic materials, offering all the advantages of molecular chemistry (room temperature synthesis, purity, solubility in many solvents, a well defined periphery of organic groups, a crystalline ensemble of monodisperse units) as well as displaying the superparamagnetism of a mesoscale magnetic particle. They also display quantum tunneling of magnetization (QTM), emphasizing that they straddle the interface between the classical and quantum regimes. SMMs have many potential applications, but these require that their properties be both understood and controlled, particularly QTM. The Mn12 SMMs are the best understood. Various derivatives have been prepared differing in the organic groups, and it has been discovered that the magnetic properties (including QTM) can be significantly altered. This is also possible by adding additional electrons, and both the [Mn12]- (S = 19/2) and [Mn12]2- (S = 10) versions have been prepared. Mn4 SMMs with S = 9/2 have also been extensively studied. In some cases, two Mn4 SMMs occur as supramolecular dimers, [Mn4]2, and exchange interactions between them lead to interesting modifications of their QTM properties, establishing the feasibility of tuning the QTM in SMMs.[2]

[1] G. Christou, D. Gatteschi, D. N. Hendrickson, and R. Sessoli, MRS Bulletin 25, 66 (2000).
[2] W. Wernsdorfer, N. Aliaga-Alcalde, D. N. Hendrickson, and G. Christou, Nature 416, 406 (2002).

2:40pm **MB+BI+OF-TuA3 Density-Functional-Based Simulation of Molecular Magnets**, ***M.R. Pederson***, *N. Bernstein*, Naval Research Laboratory, *T. Baruah*, Georgetown University, *J. Kortus*, Max-Planck-Institute, Germany **INVITED**

Recently a class of transition-metal containing molecules have attracted significant experimental interest because they retain their magnetic orientation at relatively high temperatures and because they exhibit quantum tunneling of magnetism. These molecular magnets consist of approximately 70-200 atoms and are typically composed of 4-15 transition metal atoms which are held in place by organic ligands and anions. The fundamental figure of merit which governs these phenomena is the magnetic anisotropy which arises due to the spin-orbit interaction and other couplings between spin and spatial degrees of freedom. Recently, a quantum-mechanical method has been developed which allows for the density-functional-based determination of magnetic anisotropies in molecules and clusters.[1] We have used this method to calculate anisotropies in several molecular magnets which include: $Mn_{12}O_{12}(RCOO)_{16}(H_2O)_4$, $Fe_8O_2(OH)_{12}(C_6N_3H_{15})_6$, $Co_4C_5NH_4CH_2O)_4(CH_3OH)_4Cl_4$, and $[Mn_{10}O_4$(2,2'-biphenoxide$)_4Br_{12}]^{4-}$. Our calculations show that good agreement between experiment and theory can be obtained. While the reorientation barriers and magnetic resonant tunneling fields are primarily determined from the second-order anisotropy hamiltonian,[1] higher-order effects can change these quantities by about ten percent. Further, such effects determine tunnel splittings and play a significant role in tunneling dynamics. Currently the primary source of such splittings is an active area of investigation. We have recently suggested that vibrationally induced changes in the spin-orbit interaction will contribute to higher-order anisotropies.[2] Further, computational results on the 4th-order magnetic anisotropy show that this interaction may provide a dominant contribution to the higher-order barriers and that it partially contributes to tunnel splittings. We discuss these calculations and compare our results to the experimental infrared work of Sushkov et al which shows that certain vibrational intensities are strongly perturbed by applied magnetic fields in the Mn_{12}-Acetate system.[3] A very brief review of the computational method, NRLMOL, used in this work will be included in the talk.

[1] M.R. Pederson and S.N. Khanna, Phys. Rev. B 60, 9566 (1999).
[2] M.R. Pederson, N. Bernstein and J. Kortus, (Cond-mate/0201353).
[3] A.B. Sushkov, B. Jones, J.L. Musfeldt, et al, Phys. Rev B 65,(2002).

3:20pm **MB+BI+OF-TuA5 Measuring and Manipulating Single Molecules Inside Living Cells**, ***J.S. Kanger***, *A.H.B. de Vries, J. Greve,* University of Twente, The Netherlands, *B. Krenn, R. van Driel*, University of Amsterdam, The Netherlands **INVITED**

For manipulating single molecules, techniques like AFM or optical tweezers are typically used. However, the actual actuators of these systems are relatively large, and therefore we are not able to manipulate single molecules that are situated deep inside the cell (for example inside the nucleus), without causing massive damage to the cell itself. We describe a conceptual simple arrangement for manipulating ultra small magnetic beads inside living cells using magnetic forces. By using magnetic forces to manipulate the bead, and a low yield HeNe laser to measure its position, we are able to generate a relatively high force, without damaging the cell. The setup is designed to measure the movement of a bead with nanometer precision, and apply picoNewton forces on it. Experimental results combined with model calculations show that a force of 15 pN is feasible for a ferrite bead of 50 nm diameter. If a bead is attached to a functioning protein the movement of this protein in the cell can be monitored and manipulated. We plan to apply this technique to the study of chromatin structure function relations inside the living cell. The magnetic force on a bead is proportional to the magnetization of the bead, and the gradient of the magnetic field. To produce a magnetic field that gives a gradient that is controllable both in direction and strength we constructed a four pole configuration. The tips of these poles (5 µm width and height) are placed 20 µm from each other, which leaves enough space to place a cell, with a magnetic bead in the nucleus, between the poles. The magnetic field is guided from external coils to the poletip that becomes magnetically saturated (1.8 Tesla). The pole tips are produced in the cleanroom facilities of our university. Bead position detection is done by back focal plane interferomtery. A low-yield HeNe laser will is focused on the bead. The combination of the laserbeam and, and the forward scattered light gives a interference pattern on a quadrant detector, which is depended on the position of the bead in the focus.

4:00pm **MB+BI+OF-TuA7 Synthesis and Functionalization of Nanoparticles**, ***A. Ulman***, Polytechnic University **INVITED**

The talk will focus on metal and metal oxide nanoparticles. A one-phase synthesis of thiolate-functionalized metallic nanoparticles will be described, and further chemical reactions, such as surface-initiated polymerization and attachment of DNA bases will be presented. Sonochemical preparation of oxide and mixed oxide nanoparticles will be reported. We have demonstrated, for the first time, that sonication is a very efficient method for coating of γ-Fe_2O_3 and other oxide nanoparticles. The attachment of enzymes to γ-Fe_2O_3 nanoparticles will be described.

Biomaterials
Room: Exhibit Hall B2 - Session BI-TuP

Biointerfaces and Surfaces I

BI-TuP1 Computer Simulation of Water Near Structureless Model Surfaces and Self-assembled Monolayers: Interfacial Behavior and Hydration Forces, ***T. Hayashi***, *A.J. Pertsin, M. Grunze*, Heidelberg Universität, Germany

The hydration forces between both structureless and structured surfaces are calculated using the grand canonical Monte Carlo technique. Primary attention is given to large surface-to-surface separations (40 Å and more), where the oscillations of the hydration force have practically decayed. For simulations of structureless surfaces, both orientation independent and strongly directional potentials are employed. Our results show that water confined between hydrophobic surfaces experiences a capillary evaporation at surface-to-surface separations less than about 58 Å. At larger separations, hydrophobic attraction due to a density depression in the confined region is observed. In cases of hydrophilic surfaces, the sign and magnitude of hydration force are found to be strongly dependent on the presence of orientation dependent terms in the water-surface potential. Simulations of structured surfaces were performed with methoxy tri(ethylene glycol) terminated alkane thiol self-assembled monolayers (SAMs) on the Au(111) and Ag(111) substrates. Although both of the SAMs show a typical hydrophobic behavior similar to that observed with structureless hydrophobic surfaces, there are substantial differences in their interaction with water. The simulation results are discussed in the context of the experimentally observed protein adsorption properties and surface force behavior between the SAMs.

BI-TuP2 Permittivity Responsive Interface for Biosensor in Tissue Engineering, ***P.O. Bagnaninchi***, *M. Tabrizian*, Mc Gill University, Canada

We have designed micro-porous host medium which fit with requirements for permittivity changing interface. The main application is the assessment of the growth of cells in micro-porous matrix for tissue engineering. These biointerfaces will be used with evanescent wave transducer or Inter Digital Capacitance since they are based on the change of permittivity in a surrounding medium. The complex biological medium is heterogeneous and our approach to describe the macroscopic dielectric behaviour of this mixture in terms of the bulk properties of the constituents is based on and derived from the effective medium theory; which is valid as long as the wavelength of electromagnetic wave is larger than the size of heterogeneities. Our method allow us to design permittivity responsive matrix and to improve the sensitivity of the biosensor in order to assess in a non-destructive way the growth of cells in micro-porous matrix. In this study the biointerface is characterized by complex permittivity measurement over a wide frequency range with the aid of a dielectric probe and network analyser.

BI-TuP5 Interaction of Poly(L-Lysine)-g-poly(ethylene glycol) with Negatively Charged Supported Phospholipid Bilayers, ***F. Rossetti***, Swiss Federal Institute of Technology, *I. Reviakine*, University of Houston, *G. Csucs, S.M. De Paul, J. Vörös, N.D. Spencer, M. Textor*, Swiss Federal Institute of Technology

The goal of the study presented in this poster is to develop a general, one-step method for modifying the surfaces of different types of vesicles. The proposed idea is to coat negatively charged phospholipid or polymeric vesicles with functionalised Poly(L-Lysine)-g-poly(ethylene glycol) (PLL-g-PEG), a polyelectrolyte with a positively charged backbone and protein-resistant ("stealth"[1]) side chains. Negatively charged supported phospholipid bilayers (SPBs) were used to investigate the adsorption behaviour of PLL-g-PEG as a function of charge density. The main investigation methods used were the Quartz Crystal Microbalance (QCM-D, including measurement of dissipation) and fluorescence microscopy. The SPB system used consisted of mixtures of a zwitterionic phospholipid (dioleyl phosphatidyl choline - DOPC) and a negatively charged phospholipid (dioleyl phophatildyl serine - DOPS) in a range from 0 (neutral SPB, used as a control) to 18 mol-%. Effects of the buffer composition on the adsorption process (with particular attention to the ionic strength) and the presence or absence of Ca^{2+} ions, which were found to be crucial for the formation of an SPB from DOPC:DOPS vesicles, were also considered.

[1] Woodle, M.C., Lasic D.D., Biochim. Biophis. Acta, 1992, 1113, 171-199.

BI-TuP6 Derivatization and Surface Characterization of poly(2-hydroxyethyl methacrylate) for Oriented Protein Immobilization, ***J.L. Schwartz***, *S.M. Martin, D.G. Castner, C.M. Giachelli, B.D. Ratner*, University of Washington

When biomaterials are placed in the body they are immediately covered with a random layer of mixed proteins. This may trigger the foreign body reaction leading to collagenous tissue encapsulating the biomaterial. To circumvent this, many schemes have been developed to pre-immobilize a protein or peptide onto the surface intended to subdue the inflammatory response. The goal of this study is to immobilize osteopontin (OPN) in a single orientation such that the active site is presented to the surface for optimal cellular interaction. Proteins can be immobilized via a primary amine from a lysine unit, but due to the number and distribution of lysine in OPN there is no control over the final orientation. A different approach is to immobilize OPN engineered with a polyhistidine tail, which can interact with the coordination sites of a divalent metal ion. The recombinant six-histidine (His-tag) sequence does not hinder protein activity and can be placed at a characteristic site on the protein of interest. The divalent metal ion, Ni^{2+}, binds tightly to nitrilotriacetic acid (NTA), a metal chelating agent covalently attached to poly(2-hydroxyethyl methacrylate) (pHEMA) via a N,N-carbonyldiimidazole (CDI) intermediate reaction step. The choice of pHEMA as the immobilizing substrate was due to the abundance of hydroxyl groups on the surface as well as low non-specific protein adsorption. These surfaces were characterized by XPS and ToF-SIMS before and after chemical derivatization as well as after each reaction step. Derivatization with fluorine containing molecules was used to probe hydroxyl, carboxyl, and imidazole carbamate availability with XPS. The amount of protein immobilized to pHEMA was quantified by ^{125}I radiolabeled OPN and the protein was tested for retained biological activity.

BI-TuP9 Polysaccharide Adsorption on Hydrophobic and Hydrophilic Surfaces, ***K.T. Queeney***, *C. Royce*, Smith College

The interaction of extracellular polysaccharides with solid substrates plays an important role in the adhesion of bacterial cells to a variety of natural and synthetic surfaces. We have used a combination of surface infrared spectroscopy and atomic force microscopy to investigate the fundamental chemical interactions that govern the adsorption behavior of such polysaccharides. The adsorption of xanthan gum, a model polysaccharide, is studied on silicon and silica surfaces that have been modified to present a range of both surface wettability and chemical functionality. Adsorption is monitored both in- and ex-situ, using a custom-designed cell for infrared spectroscopy at silicon/aqueous interfaces. Simultaneous studies of the adsorption characteristics of the constituent monosaccharides of xanthan provide a way to assess the importance of effects such as polymer conformation and polymer-polymer interactions in the adsorption process. For instance, while monosaccharides exhibit similar adsorption characteristics on hydrophilic, silanol-terminated silica and hydrophobic, hydrogen-terminated silicon, xanthan exhibits a marked preference for the hydrophobic surface. The importance of adsorbate-adsorbate interactions in the adsorption of both poly- and monosaccharides is explored through analysis of spectral evolution from mono- to multilayer regimes.

BI-TuP10 Plasma Treatment of Plastics to Reduce Water Adhesion and Bio-fouling, ***M.J. Neumann***, *P.J.A. Fackler, D.N. Ruzic*, University of Illinois at Urbana

Polymers have become a part of our everyday lives for use in a wide range of applications. This is due to polymers exhibiting high strength with little weight, wide range of flexibility, ease of formability, and economics of production. However, processes designed to achieve the desired surface properties of a polymer can comprimise the overall bulk material. The ability to alter the surface of the polymer while leaving the underlying bulk material unchanged has a large potential for development in the area of biomaterials. By modifying the surface of a polymeric material so as to impede water adhesion, the ability of bacterial and cell growth and hence, infection, can be minimized on those surfaces that are incorporated into biological systems. Surface modification was accomplished via plasma processing in a commerical size plasma etching device which achieves plasma densities and and electron temperatures up to 10^{11} cm^{-3} and 4 eV. The desired degree of change is controlled by macroscopic external controls, rather than invasive internal modifications. This process lends itself well for use in exisiting plasma processing systems. Water contact measurements have been taken before and after treatment of HDPE that show a change from a pretreatment of 85^{o} to post treatment of near 0^{o} and 0^{o} angles, which corresponds to a dramatic change in surface energy of the polymer. Video of the surface interaction with water shows drops rolling off

the material. Applications of the process includ intubation tubes, blood vials, shunts, splints, and many other biomedical devices.

BI-TuP11 Comparison of Polystyrene and Teflon-AF as Model Surfaces for Hydrophobic Adsorption and Biocompatibilization, ***L. Feller***, *N. Tirelli, S.M. De Paul, J.P. Bearinger, A. Napoli, J.A. Hubbell, M. Textor,* Swiss Federal Institute of Technology

The motivation of this investigation is to produce materials for applications in biosensors and biomedical materials via surface modification based on hydrophobic interactions. For this purpose we have used the physical adsorption of amphiphilic poly(propylene sulfide)-block-poly(ethylene glycol) (PPS-PEG), from a polar solvent (methanol, water). Upon deposition on a hydrophobic surface, these polymers display the biocompatible, protein-repellent PEG as the top layer and are believed to maintain this architecture when placed in either water-based model electrolytes or a physiological environment. We have chosen to apply this method of surface modification to two hydrophobic materials, poly(styrene) and Teflon-AF. These materials have already been used in biomedical applications but which require a surface biocompatibilization to reduce unfavorable foreign-body reactions. Both polymers can be produced in form of thin films (20-50 nm thickness) via spin-coating. We studied the properties of thin films of these two hydrophobic surfaces and their behavior in subsequent PPS-PEG adsorption studies. The thickness and uniformity of spin-coated surfaces are sensitive to rotation speed and concentration. For optical waveguide lightmode spectroscopy (OWLS) investigations it is necessary to have an optically transparent homogenous thin layer of ideally 12 nm thickness. We produced a series of both poly(styrene) and Teflon-AF layers of various thicknesses and characterized their homogeneity and thickness using an Atomic Force Microscope (AFM). Layer stability was characterized by dynamic contact angle measurements. The adsorption of PPS-PEG block copolymers through hydrophobic interactions was subsequently studied by OWLS. PPS-PEG demonstrated good adsorption on both surfaces, showing complete and stable coverage under physiological conditions. Preliminary experiments have also shown that the deposited layers strongly decrease the protein adsorption on such substrates.

BI-TuP12 In-situ Single-Molecular Detection of Antibody-Antigen Binding by Tapping-Mode Atomic Force Microscopy, ***L. Li***, *S. Chen, S. Jiang*, University of Washington

Ever since its invention atomic force microscopy (AFM) has been widely used in biotechnology and biomedical research, including imaging, force mapping and sensor application. In this work, we have performed studies on AFM-based single-molecule detection. Target molecules are detected by directly comparing two tapping-mode AFM topographical images at the same location before and after exposing an immobilized antibody to a solution containing its antigen, or vise visa. Two pairs of antigen/antibody systems were investigated: chorionic gonadotropin (hCG) and monoclonal antibody (MAb) to hCG, goat anti-hCG and MAb to goat immunoglobulin (IgG). Antibody molecules are chemically immobilized on uniform mixed self-assembled monolayers (SAMs) terminated with COOH and OH, which allow the detection of the individual antigens, antibodies, and antigen/antibody complexes. The advantages of the in-situ detection at the same location include the detection of antigen/antibody binding at single-molecule resolution and the distinction of non-specific interactions from specific ones. This AFM-based immunoassay is more sensitive and reliable.

BI-TuP13 Transformation of a Single Peptide Molecule Measured with Atomic Force Microscopy, ***M. Kageshima***, *S. Takeda,* National Institute of Advanced Industrial Science and Technology, Japan, *A. Ptak,* Poznan University of Technology, *C. Nakamura, S.P. Jarvis, H. Tokumoto, J. Miyake*, National Institute of Advanced Industrial Science and Technology, Japan

Transformation of protein molecule is a fundamental process in various function of the molecule. Such a transformation is considered to accompany substantial rearrangement of intramolecular hydrogen bonds. In a peptide molecule in an α-helix form, breaking of hydrogen bonds takes place as it is unfolded by a tensile force along its helical axis and results in variation in the longitudinal stiffness of the molecule. Therefore, in order to understand the unfolding process in a single-molecule scale, measurement of the variation in stiffness and the energy dissipated during refolding process is indispensable. In the present study this measurement was implemented by AFM with magnetic modulation technique. An end of a single $C_3(AEAAKA)_6C$ peptide molecule was picked up with the AFM probe and was stretched. The AFM cantilever was modulated with an AC magnetic force with a frequency of 500 Hz via a magnetic particle on its backside. The amplitude and phase shift in the AC component of the cantilever deflection were measured simultaneously with the DC force. The dissipation during one cycle of oscillation and the variation in the stiffness of the molecule was calculated from the measured amplitude and phase. The contribution by the liquid in the measured dissipation was calculated from the amplitude signal and was subtracted. From the dissipation change during the unfolding process, the dissociation energy per one hydrogen bond was determined. Thus, it is shown that both the conservative and the dissipating processes taking place in a single molecule during its transformation can be measured with this technique.

BI-TuP14 Selective Photocatalysis by Means of Molecular Recognition, ***Y. Paz***, Technion- Israel Inst. of Technology, Israel

Titanium dioxide is known to be a non-selective photocatalyst for the treatment of polluted air and water. An approach for obtaining selectivity, thus facilitating its use for the mineralization of hazardous, non-biodegradable contaminants is presented hereby. This approach is based on the construction of molecular recognition sites (MRS) anchored on inert domains in the vicinity of photoactive sites. These MRS are designed to physisorb target molecules and to "shuttle" them to the photocatalytic sites. Care is taken to prevent the photocatalytic degradation of the MRS, since (as we have found before) the photoinduced oxidizing species are, in principle, able to attack molecules anchored in the vicinity of titanium dioxide domains. Here we present several examples of selective photocatalysis by means of molecular recognition, based on the trapping of target molecules on thiolated cyclodextrins sites, followed by surface diffusion and photodegradation of the contaminants. The prospects and limitations of this approach will be discussed.

BI-TuP15 Reflex Arc on a Chip - Directed Neuron Growth, ***M. Poeta***, *G. Jacob, M. Das, P. Molnar, J. Hickman,* Clemson University

The reflex arc is one of the simplest controls systems in the body. Yet it rivals the most complex man-made systems in complexity. The reflex arc is a controls loop consisting of a muscle fiber innervated by a motoneuron. A dorsal root ganglion (DRG) innervates both cells, completing the loop and providing feedback. Our group will look at building this system on a Micro Electrical Mechanical System (MEMS) chip. Currently, we are investigating the directed growth of motoneurons on substrates. We have created patterns of Self Assembled Monolayers (SAMs) on glass cover slips. The patterns have two geometric variables: somal (cell body) adhesion site diameter and axon (signal sending process) track width. The patterns are made of diethylenetriamine (DETA), a SAM cytophilic to motoneurons. The cover slips were then backfilled with tridecafluoro-1,1,2,2-tetrahydrooctal-1-trichlorosilane (13F), a SAM cytophobic to motoneuron growth. Photolithographic techniques are used to create the patterns. We are determining the geometric parameters (somal adhesion site diameter, axon track width) that are most conducive to motoneuron growth. We also will present electrophysiological characteristics of the motoneurons and relate changes in electrical activity to parameters in the neuron local environment.

BI-TuP17 Explorations of the Influence of Electrostatic Interactions on Surface-Peptide Binding by Matrix-Assisted Laser Desorption / Ionization Mass Spectrometry, *G.R. Kinsel,* ***J. Zhang***, *R.B. Timmons, M. Li*, University of Texas at Arlington

Protein-surface interactions play an important role in a variety of fields. The mechanism of these interactions remains unclear, however, due to the extraordinary complexity of the protein-surface interface and the wide range of chemical and morphological properties that may be present. The use of well characterized surfaces and peptides with well-defined properties can alleviate some of these problems and allow the systematic study of the influence of various surface or protein properties on the protein-surface binding interactions. In the present studies surfaces having well characterized chemical and morphological properties have been created by plasma polymerization of allyl amine or vinyl acetic acid leading to surfaces with high contents of amine or carboxylic acid functional groups respectively. Bradykinin, angiotensin I, and buccalin are three small peptides with similar molecular weights but various primary sequences leading to systematic changes in the peptide pI from 12.0 to 6.9 to 3.8 respectively. It is shown that these peptides have increasing binding affinity for plasma polymerized vinyl acetic acid modified PET surfaces, but decreasing binding affinity for plasma polymerized allyl amine modified PET surfaces. These trends may be attributed to electrostatic interactions between the peptides and the chemical groups on the plasma modified surfaces. This interpretation of the observed effects can be further explored by altering the pH of the solution in which the binding interactions take place. For example, it is found that as the acidity of the solution is increased binding of the acidic peptide to the basic surface is reduced, consistent with the peptide being neutralized in low pH solutions. Additional studies that explore the effect of solution pH on peptide surface binding interactions have been performed and are interpreted in terms of the changing electrostatic properties of the peptide and surface.

BI-TuP18 Realisation of Biosensor Interfaces by Surface Reactions on Silanised Tantalum Pentoxide, ***W. Laureyn***, *R. De Palma, F. Frederix, K. Bonroy, J.-M. Friedt, K.-H. Choi, A. Campitelli,* IMEC, Belgium, *G. Maes,* KULeuven, Belgium

Affinity biosensors allow the detection of affinity based interactions between bio-molecules, e.g. in antibody-antigen recognition. The presence of antigens in an analyte can be verified by their binding to complementary antibodies, immobilised onto a biosensor surface. Tantalum pentoxide (Ta_2O_5) is regarded as a promising material for the realisation of affinity biosensors, especially for impedimetric biosensing, because of its high dielectric constant and chemical stability. To date, the main method for the immobilisation of proteins to oxide surfaces has involved reactions with short-chain trialkoxysilanes, leading to heterogeneous and less effective biosensor interfaces. Alkyltrichlorosilanes, on the contrary, generate well-structured Self-Assembled Monolayers (SAMs), when produced under the proper conditions. Unfortunately, most polar functional groups ideal for protein immobilisation (COOH and NH_2) have to be generated from non-polar precursor alkyltrichlorosilanes, after SAM-formation of the latter. In this contribution, several approaches for the introduction of polar functional groups on Ta_2O_5 , silanised with alkyltrichlorosilanes, will be presented. A novel surface reaction for the introduction of COOH and NH_2 groups on SAMs of bromoalkyltrichlorosilane is evaluated and compared to the oxidation of allylalkyltrichlorosilane and the reduction of cyanoalkyltrichlorosilane respectively. The proposed surface reaction consists in a nucleophilic substitution of the bromine termination with functional thiol compounds. The silanisation of Ta_2O_5 and the subsequent surface reactions are characterised by means of contact angle measurements, XPS, infrared spectroscopy and cyclic voltammetry. Finally, the immobilisation of IgG, on the generated functional Ta_2O_5 surfaces, and the subsequent binding of anti-IgG are monitored by means of Quartz Crystal Microbalance and Atomic Force Micrososcopy.

Biomaterials
Room: C-201 - Session BI+AS-WeM

Ambient Surface Science Techniques
Moderator: M. Grunze, Heidelberg Universität, Germany

8:20am **BI+AS-WeM1 A Challenging Problem: Interfaces between Condensed Matter**, ***M. Buck***, StAndrews University, UK **INVITED**

Surface Science has developed a zoo of techniques which allow the characterization of chemistry and structures of surfaces and adsorbates at an impressive level of molecular detail. Unfortunately, the large variety of available techniques dramatically reduces when dealing with systems under non-vacuum environment and, therefore, an understanding on a molecular level is much harder to gain. In addition to problems on the technical side, the situation is further complicated by the fact that "real world" interfaces are, in general, more complex compared to systems studied in surface science, e.g. larger molecular entities with more conformational degrees of freedom, amorphous structures, and additional interactions due to the environment. The talk discusses various routes to unravel the relationship between structures and properties of biomaterials interfaces and highlights problems and possible pitfalls associated with the investigation of such type of interfaces.

9:00am **BI+AS-WeM3 Surface Chemistry of Environmentally Relevant Transition Metal Oxides Studied in Aqueous Solutions using Soft X-ray Spectromicroscopy**, ***B.P. Tonner***, *K. Pecher*, University of Central Florida

The surface chemistry of environmentally relevant inorganic oxides can now be reliably assessed in solution, with high spatial resolution, using a methodology based on x-ray absorption spectroscopy with microfocussing.[1,2] A crucial aspect of this research is that the studies are performed in the presence of a complete water layer, with control of parameters such as buffer concentrations, dissolved oxygen content, and pH. We have concentrated on the fate of Fe and Mn oxides in mineral model compounds, and in addition important nano-scale materials like the 'green rusts.' Spatial chemical inhomogeneities are prevalent in such nano-scale minerals, and are revealed by x-ray spectro-microscopy "chemical state mapping." The state of these studies has matured to the point where chemical intermediates, formed as a result of microbial metabolism, can be reliably detected and identified. This paper will emphasize the quantitative aspects of performing assays of surface transition metal oxide valence distributions using L-edge spectromicroscopy.

[1] Rothe, J., E.M. Kneedler, K.H. Pecher, B.P. Tonner, K.H. Nealson, T. Grundl, W. Meyer-Ilse, and T. Warwick, Journal of Synchrotron Radiation 6, 359-361 (1999).

[2] K. Pecher, E. Kneedler, J. Rothe, G. Meigs, T. Warwick, K. Nealson, and B. P. Tonner, X-ray Microscopy 1999, W. Meyer-Ilse, T. Warwick, and D. Attwood, ed., (American Institute of Physics, NY, 2000) p. 291-300.

9:20am **BI+AS-WeM4 Investigation of Protein Adsorption with Simultaneous Measurements of Atomic Force Microscope (AFM) and Quartz Crystal Microbalance (QCM)**, ***K.-H. Choi***, *J.-M. Friedt, F. Frederix, W. Laureyn, A. Campitelli, G. Borghs*, IMEC, Belgium

We have combined the tapping mode atomic force microscope (AFM) and quartz crystal microbalance (QCM) for the direct investigation and characterization of protein adsorption on various metallic surfaces. The adsorption of proteins, such as human plasma fibrinogen, γ-globulin and collagen, onto the metal/QCM surface were monitored using both methods at the same time when varying the concentration of them. We present the AFM images that shows the surface changes and the adsorption scheme of proteins with molecular resolution according to the shift of resonant vibration frequency of the QCM. The combination of AFM with QCM and the simultaneous measurements of the bio molecule adsorption with two techniques provide us with not only the sensing and detection technique but also the means for understanding the adsorption schemes of bio molecules on the metal surface.

9:40am **BI+AS-WeM5 Real-time AFM Investigations of the Enzymatic Degradation of DNA-polymer Dendrimer Complexes**, ***S.J.B. Tendler***, *H.G. Abdelhady, C.J. Roberts, S. Allen, M.C. Davies, P.M. Williams*, University of Nottingham, UK

Fundamental to surface recognition strategies is the need to develop both interfaces and imaging methods that allow the investigation of biomolecular recognition processes in solution, in-real time. One such set of processes is the enzymatic degradation of DNA, both when naked and when protected by polymeric (bio)materials. This system has clinical relevance in that polyelectrolyte complexes between polyamidoamine (PAMAM) dendrimers and DNA have emerged as potential non-viral vectors for therapeutic DNA delivery. Hence methods for analyzing the ability of PAMAM dendrimers to protect the DNA from degradative enzymes are of clinical significance. Here we have applied atomic force microscopy (AFM) in liquid to visualize at the molecular scale and in real time, the effect of the enzyme DNase I on generation 4 PAMAM dendrimers complexed with DNA (G4-DNA). The formation of G4-DNA is observed to provide a degree of protection to the DNA, the level of which rises with increasing PAMAM dendrimer to DNA ratio and to a certain degree with the time allowed for complexes to form.

10:00am **BI+AS-WeM6 Interaction of Water with Protein Resistant Self-Assembled Monolayers: Neutron Reflectivity Measurements of Water Density in the Interphase Region**, ***D. Schwendel***, *T. Hayashi, A.J. Pertsin, R. Dahint*, University of Heidelberg, Germany, *R. Steitz*, Hahn-Meitner-Institut, Germany, *F. Schreiber*, University of Oxford, UK, *M. Grunze*, University of Heidelberg, Germany

The interfacial behavior of surfaces, colloids, and molecules with water plays a substantial role in surface science and other areas. It is, in particular, responsible for colloid stability, micelle formation, biomembrane fusion, and the resistance of materials against proteins from biological media. These materials are of crucial importance in biotechnology and biomedical applications. One type of such bicompatible surfaces is represented by self-assembled monolayers (SAMs) on Au and Ag composed of undecanethiolates terminated oligo(ethylene glycols), (-O-CH2-CH2-)n (hereafter EGn). Neutron reflectivity measurements on protein resistant methoxy tri(ethylene glycol) (EG3-OMe) and hydroxy terminated hexa(ethylene glycol) (EG6-OH) undecanethiolate self-assembled monolayers (SAMs) in contact with deuterated water reveal the presence of an extended (~5 nm thick) water interphase with a noticeably reduced density (85-90 % of bulk water density). This result is in qualitative agreement with Grand canonical Monte Carlo simulations of water next to the SAM surface. For comparison, neutron reflectivity experiments have also been performed on non-functionalized hydrophobic octadecanethiolate and hydrophilic hydroxy terminated undecylthiolate SAMs. Additionally, neutron reflectivity measurements on protein resistant SAMs formed from hydroxy and methoxy terminated tri(ethylene glycol) (EG3-OH and EG3-OMe) against high concentrated protein solutions of BSA show that the free dissolved protein does not contact the surface but that it is repelled over a distance of few nm. The profiles strongly suggest a BSA depleted water layer at the SAM/bulk interface of 4 to 6 nm while BSA adsorption is observed for non-resistant propoxy terminated tri(ethylene glycol) (EG3-OPr).

10:40am **BI+AS-WeM8 Force Spectroscopy of Self-Assembled Monolayers Containing 'Sandwiched' Oligo(Ethylene Glycol) Interfaces on Gold under Electrolyte Solution**, ***G. Haehner***, *C. Dicke*, University of St Andrews, UK, *S. Herrwerth, W. Eck, M. Grunze*, University of Heidelberg, Germany

Non-specific interactions between biomolecules and (synthetic) organic surfaces, and in particular materials which are resistant to the adsorption of proteins from biological media, are of crucial importance to the fields of biomaterials, biosensors and medical devices. Chemically functionalized (charged and hydrophobic) scanning force microscope probes can mimic local structures of proteins and hence allow it to study the influence of these parameters on the overall observed interaction separately. Oligo(ethylene glycol) (OEG) terminated self-assembled monolayers on gold show high inertness towards the non-specific adsorption of proteins. The underlying mechanism, however, has not yet been resolved completely. It appears that water as well as hydronium and/or hydroxyl ions play a central role. In order to scrutinize the interaction, the accessibility of the OEG interface to molecules/ions from solution was varied. This was accomplished by the molecular structure: the functional (OEG) part was terminated with hydrophobic chains of different length resulting in 'sandwich'-film-structures. Force spectroscopy measurements on these layered structures with hydrophobic probes under electrolyte solution reveal the importance of the different contributing factors to the overall interaction.

Biomaterials
Room: Exhibit Hall B2 - Session BI-WeP

Biointerfaces and Surfaces II

BI-WeP1 Bioactive Surfaces for Control of Stem Cell Differentiation, ***J. Kelly***, *D. Dahlborg, S. Svedhem, D. Sutherland,* Chalmers University of Technology, Sweden, *P. Eriksson,* Sahlgrenska University Hospital, Sweden, *J. Gold,* Chalmers University of Technology, Sweden

Tissue engineering of the peripheral and central nervous systems stands to make great progress if the regenerative potential of the recently isolated neural stem cells can be harnessed and directed by the use of synthetic materials and constructs. We have produced surfaces with bound stimulatory molecules (proteins and growth factors) to control neural progenitor cells from the hippocampus of adult rats (AHPs) which have the ability to regenerate the progenitor phenotype or differentiate down one of two possible lineages to become neuron- or glial-like cells. The stimulatory molecule of interest may be patterned on surfaces with high spatial resolution by microcontact printing with a stamp fabricated by casting polydimethyl siloxane on a master with a negative of the desired pattern. We have combined this initial biopatterning step with either adsorption of a second, co-active protein or with a supported lipid bilayer (SLB). SLBs are membrane-like thin films which form by fusion of lipid vesicles on SiO2 or glass surfaces. Unmodified SLBs are resistant to protein adsorption and cell adhesion thus offering the ability to pattern areas of adhesive and unadhesive character. Moreover by inclusion of modified lipids we functionalised a SLB with a neuroactive 19-mer IKVAV sequence derived from laminin, via a maleimide coupling. This surface promoted high levels of cell attachment and presented an otherwise non-perturbing background to cells and proteins. We have assessed the biological activity of several proteins for control of cell function and lineage, including laminin, ciliary neurotrophic factor (CNTF) and fibroblast growth factor (FGF-2) by adsorption or printing on glass surfaces. Laminin supported a mixed population of proliferative and differentiated cells. AHPs on CNTF differentiated to glial phenotype as shown by expression of glial fibrillary acidic protein while on FGF-2, cell proliferation was maintained without differentiation.

BI-WeP2 Oriented Immobilization of Anti-Human Chronic Gonadotropin for Biosensor Applications, ***L. Liu***, *S. Chen, S. Jiang*, University of Washington

The orientation of immobilized antibodies on solid surfaces, is important to performance of a biosensor. Our previous studies show that it is possible to control antibody orientation during physical adsorption via adjusting surface and solution properties. For biosensor applications, it is desirable to chemically immobilize antibody molecules on surfaces. Our previous studies further show that chemical linkers (e.g., NHS/EDC and GLU) used for chemical immobilization alter surface charges, thus antibody orientation. In this work, we present a new method, which combines the site-directed immobilization (via the conservative carbohydrate or histidine rich region located in the Fc portion of the IgG) and the charge-control methods, for the oriented immobilization of antibodies on self-assembled monolayers (SAMs)/Au(111). Monoclonal anti-hCG (human chronic gonadotropin) will be a model antibody studied in this work. Surface charge is adjusted by changing SAM terminal groups and solution pH values. Atomic force microscopy (AFM) is used to characterize adsorbed antibody molecules on surfaces. Low detection limit and saturated adsorbed amount in surface plasmon resonance (SPR) biosensors are determined in this work for various antibody immobilization methods. Results from this work show significant improvement over those based on conventional immobilization methods.

BI-WeP3 Protein Absorption in Engineered MEMS Test-beds, ***D. Henry***, *K. Lenghaus, U. Jalgaonkar, J. Dale, J.C. Henderson, J. Hickman,* Clemson University

We are studying the influence of surface modification, channel geometry, flow conditions, etc., on protein absorption. The information found from these experiments will help create new knowledge for developing biocompatible MEMS systems. To this end we have developed a test system that involves clamping two silicon wafers together that have half of a channel or device etched in them, which allows for separation and examination of the wafer surface after the enzyme flow experiments have been conducted. The enzyme absorption to the channels can thus be investigated by standard enzyme assays as well as surface analysis directly in the channel halves. The primary enzymes used in this study include alkaline phosphotase, glucose oxidase, and TAQ polymerase. We have engineered a working version of this system, however, during development of the clamping system a problem arose with fluid leaking over and out of the channels. We hypothesized this was because the surface of the silicon wafer is hydrophilic and the fluid was drawn between the wafers rather than just flowing through the channels. Our results will present the solution to this problem via hydrophobic surface modification on the interior face of the silicon wafers. We will also present results on the development of the system, our experiment to optimize it, its application to determine how the proteins bind in the channels, where they bind in the channels, and if there is a difference in binding between angled and straight channels.

BI-WeP4 Experimental and Modeling Results for Protein Interactions with MEMS Devices, ***F. Wang***, *J. Hickman, R.A. Latour,* Clemson University

Microelecromechanical systems (MEMS) for biomedical devices and applications generally are used to interact with simple and complex biological fluids. Undesirable biomolecular surface adsorption, which causes channel plugging, is a leading factor in the failure of such systems. We are attempting to characterize these interactions using a combination of experimental analysis and a computational fluid dynamics (CFD) model. The adsorption process includes transport of biomolecules onto the surface and the adsorptive reaction at the surface. We model the transport by Navier-Stokes equations, which accounts for the mass, momentum and energy conservation for the flow; and the adsorptive reaction is modeled by a modified Langmuir adsorption isotherm. These two parts are integrated by incorporating the surface adsorption kinetics into the transport equation as a boundary condition. A commercially available CFD code, CFD-ACE+, based on a finite volume method is employed as a starting point. BioOpter, a peripheral to this code, has been developed to extract kinetic parameters by minimizing the least-square difference between the simulation and experiment data. We will present preliminary optimization results for experiments in which a 60ng/mL Alkaline Phosphatase solution was passed through a polyethyletherketones(PEEK) modified capillary (65 microns diameter by 100 mm long) at flowrate of 0.1mL/hr which gave the adsorption/desorption parameters as: $Ka=7.2 \times 10^{5}$/m-s and $Kd=2.1^{-5}$/s. An optimization error surface model was used to visualize the location of the optimal parameters.

BI-WeP5 Fabrication and Characterization of Regular Porous Polymer Films for Biomedical Devices, ***M. Tanaka***, *M. Takebayashi, K. Sato, M. Miyama, K. Nishikawa, J. Nishida, M. Shimomura*, Hokkaido University, Japan

Porous polymer matrices are widely used in biomedical applications such as tissue engineering and artificial organs. The present studies describe the fabrication and characterization of highly regular porous polymer films formed by simple casting technique. The micro porous films were fabricated from biodegradable polymer such as poly(ε-caprolactone), poly(lactic acid-co-glicolic acid), poly(3-hydroxybutylate) and poly(L-lactic acid). Various experimental factors affecting pore size and thickness of the film, solvent, cast volume and so on, were studied. The porous film shows a highly regular hexagonal arrangement of holes in a large area and can be easily peeled off from a glass substrate as a self-supported porous film. The pore size can be controlled in the range from 1 to 50 μm by changing the evaporation time of the polymer solutions. The thickness of the film becomes thinner with decreasing the concentration. The pores were connected to each other in the porous film. The films with 1-5 g/L of concentration of the polymer had the penetrated structure. On the other hand, the un-penetrated structure was formed when the concentration of the polymer exceeded 5 g/L. The porous film with controlled pore size is used for cell separation and biomedical devices.

BI-WeP6 Patterned Construction of Three-dimensional Neuronal Networks Using Ink Jet Directed Layer-by-Layer Deposition, ***T. Xu***, *M. Das, J. Hickman, T. Boland*, Clemson University

The ability of building three-dimensional constructs for tissue engineering applications has many obvious advantages. In particular, the outgrowth and regeneration of neurons depend on a three dimensional matrix of growth factors. We present here the use of an inkjet-based system to generate three-dimensional patterns for directing neuron growth. Using a home built inkjet printing system, a mixture of collagen and poly-D-lysine was printed with a pre-designed pattern onto a glass surface that was pretreated with agarose. Primary rat E18 hippocampal neurons were cultured on the surface and allowed to attach to the pattern. After 24h incubation, a second layer of the mixture of collagen and poly-D-lysine was printed on the surface. The hippocampal neurons were again seeded on the surface and allowed to form

a second layer. This was achieved by a fixing and locking mechanism built into the printer. Communication between the different layers of the hippocampal neurons was tested and results of electrophysiological tests will be presented. The layer-by-layer approach proved successful in 3D network design and may have many other applications in tissue engineering applications.

BI-WeP7 A Novel Surface Chemistry Platform for Biochips and Bioanalytical Devices, ***M.J. Lochhead***, *S. Metzger*, Accelr8 Technology Corporation

Biochips, biosensors, and other advanced bioanalytical devices require exquisite control of biomolecular interactions with surfaces. Specificity, signal to noise ratios and detection limits of these systems are often limited by surface non-specific binding, particularly in protein-based applications. Inhibition of non-specific binding is thus a critical performance feature in the design of improved synthetic materials that contact and operate in biological fluids. While non-specific binding to surfaces is most often undesired, specific biomolecule, particle or cell binding at surfaces often is desired. The goal is to bind only one type of molecule, particle, or cell, and to do so in a manner that preserves its recognition activity and native structure. We have developed a suite of functionalized surface coatings - OptiChem - that demonstrate both low non-specific binding and robust specific biomolecule attachment. OptiChem coatings can be applied to virtually all materials commonly used in bio-analytical devices including glass, silicon, and several plastics. The coatings are organic films that combine a low binding matrix with functional groups that provide for covalent attachment or affinity binding. Reduction of non-specific binding and control of reactive group density translates into increased signal to noise ratios, thus improving upon conventional surface chemistries resulting in faster assay turnaround and lower consumption of valuable or rare samples. The capacity for coating various substrates, ready scale-up of convenient fabrication and low preparation costs make the surface chemistry ideal for many microarray applications.

BI-WeP8 New Diazonium-Functionalized Support for Fabrication of Protein Microarrays, ***Y. Wu***, *G.P. Lopez*, University of New Mexico

Microarray technologies have rapidly become a major trend in high-throughput functional genomic study since its birth at the early 1990s. Recent advances of this technology have been focused on high-throughput proteomic analysis. The difficulty in immobilizing proteins onto solid surfaces without denaturation has led to the search for new general methods for coupling proteins to solid substrates. To this end, a chemical process for covalently linking proteins onto an ordinary microscope slide in a manner that preserves the ability of the immobilized proteins to interact with other proteins has been studied. The method uses p-aminophenyl trimethoxysilane (ATMS) /diazotization chemistry that was previously developed for formation of DNA microarrays. Preliminary results showed that protein microarrays fabricated on ATMS/diazotized surfaces produced enhanced levels of protein-protein interaction, low background fluorescence and high selectivity. Orientation of the immobilized proteins on the surfaces was also studied. In addition, the antigen-antibody reaction data has been analyzed quantitatively and successfully correlated with solution concentrations. In general, this method allows binding of protein onto a solid substrate that can lead to considerable improvements in antibody-antigen interaction, stability of affixed biomolecules, and preferable protein orientation.

BI-WeP9 Multi-Electrode Arrays Surface Modification by Aligned Microcontact Printing, ***W. Wang***, *M. Poeta, J. Hickman, T. Boland*, Clemson University

Multi-electrode arrays are widely used for neuron stimulating and recording. However, such stimulating and recording efforts are most efficient when neurons are placed precisely on the electrode sites. Microcontact printing is a versatile method allowing spatially resolved surface modification and has hence been used frequently to provoke cell attachment and spreading onto engineered patterns. Microcontact printing may have applications in forming artificially designed neuronal networks in vitro. In the present study, we use microcontact printing to modify the surface of a multi-electrode array with the aim of attracting neurons to only grow on the electrode areas of the arrays. We will present several methods to fabricate stamp replicas of the electrode arrays, and to align the stamps and the multi-electrode arrays surface. We achieve this by using micromanipulators to adjust the stamp and multi-electrode arrays and patterning under a microscope. As a result, the deviation between the patterned position and the electrodes are less than a few micrometers. The usefulness of this method of creating cell arrays will be presented.

BI-WeP10 Temperature-Responsive Polymer Coatings by Plasma Polymerization and Applications for Protein and Cell Patterning, ***X. Cheng***, *Y. Wang, Y. Hanein, K. Bohringer, B.D. Ratner*, University of Washington

The thermo-responsive polymer, poly (N-isopropylacrylamide) (pNIPAM), is of great interest for research and industrial applications in separation, controlled release, tissue engineering, sensor technology, etc. In this study, RF-plasma deposition is used to create temperature responsive ppNIPAM (plasma polymerized NIPAM) coatings. Films with a good retention of the monomer side-chain functionality are produced using low power continuous plasma deposition. Protein adsorption on the coating is studied at below and above the lower critical solution temperature (31°C) using both 125I-proteins and SPR. Dramatic increases of fibrinogen, IgG and BSA adsorption (8 fold, 8 fold and 10 fold respectively) are demonstrated at 37°C on ppNIPAM films compared to the adsorption at room temperature. Proteins adsorbed at 37°C do not detach two hours after switching the incubation temperature to 23°C, in contrast to the reported reversible cell detachment from pNIPAM upon temperature drop. Antibodies adsorbed on ppNIPAM at 37°C remain functionally active, as demonstrated by SPR studies. The coating has been deposited on micro-heater arrays. The individual micro-heaters control the phase transition of the ppNIPAM directly on top of it. Spatially controlled protein adsorption on the array has been visualized through a fluorescent marker. Based on preferential adhesion of cells to certain proteins, cells can be patterned on the protein arrays and used for tissue engineering applications.

BI-WeP11 Silicone Transfer during Microcontact Printing, ***K. Glasmästar***, *J. Gold, A.-S. Andersson, D. Sutherland, B. Kasemo*, Chalmers University of Technology, Sweden

Microcontact printing, muCP, is a widely used technique for fast and low-cost micropatterning of large surface areas. Within the field of biointerfaces it is routinely used to directly pattern SAM's and proteins or indirectly control cell adhesion and growth. During microcontact printing a stamp of an elastomer, typically PDMS, inked with molecules of interest, is brought into contact with the substrate and then removed, leaving a pattern of the "ink" on the surface of the substrate. Several reports have indicated that PDMS can be transferred to the substrate under particular conditions. However, this issue has earned surprisingly little attention so far. We have systematically studied the transfer of PDMS to the substrate during muCP. XPS, ToF-SIMS and water condensation patterns were used to identify and measure the transfer. Stamps were cast from Sylgard 184 silicone elastomer (Dow Corning). Stamps were used without further treatment or after UV/ozone treatment and no external force was applied during stamping. Significant amounts of PDMS were transferred from non-treated stamps during muCP under the model conditions used. The XPS results showed that the transfer of PDMS onto both Ti and Au was significantly lowered by UV/ozone treatment of the stamp. ToF-SIMS of Au samples stamped with flat stamps confirmed the XPS results. However, the use of a patterned stamp (5 µm lines, 15 µm space) transferred more silicone to Au than a flat stamp, and UV/ozone treatment appeared to be less effective in reducing PDMS transfer. In this work we show that the UV/ozone treatment of PDMS stamps before printing lowers the amount of silicone transferred to the substrate. Oxygen plasma treatment of the stamp is likely to have the same effect. It is important to consider the potential for transfer of PDMS onto substrates when using muCP to pattern SAM's or biological molecules for biointerface applications.

BI-WeP12 X-Ray PhotoEmission Electron Microscopy of Micro-contact Printed Protein and Polymer Coated Surfaces, ***C. Morin***, *A.P. Hitchcock*, McMaster University, Canada, *D.G. Castner, B. Wickes*, University of Washington, *A. Scholl, A. Doran*, Lawrence Berkeley National Laboratory

Synthetic biomaterials are widely used in medical applications. However, their interaction with the body is mediated through passive adsorption of a disorganized adsorbed protein monolayer. Mis-recognition of this adsorbed disorganized protein layer by surrounding cells may lead to the classic foreign body reaction and device encapsulation.[1] Next generation biomaterials, or 'engineered biomaterials', are being designed in which the surface contains specific bio-recognition moieties which control the biological response of the host. Microcontact printing (µCP) is one such method which can deposit biological signalling agents with spatial resolution and fidelity.[2] It uses an elastomeric template to transfer protein molecules to a surface of interest. µCP is combined with thiol-Au self assembly to form patterns on surfaces. We are exploring the use of X-Ray photoemission electron microscopy[3] to monitor methods for preparing patterned functionalized biomaterial surfaces, and to investigate the specificity of the interaction of model surfaces with key proteins. To test the reliability of the surface patterning method, we use highly specific bio-recognition pairs, such as the biotinylated ferritin-streptavidin couple, to

probe the quality of the patterned surface. Such structures are then investigated with elemental (Fe 2p) and molecular (C1s and N1s) speciation using NEXAFS microscopy recorded at the ALS BL 7.3.1 X-PEEM. This work is supported by research and partnership grants from NSERC (Canada) and a Canada Research Chair (APH). NESAC/BIO (DGC) is supported by NIH grant RR-01296 from the National Center for Research Resources. ALS is supported by U.S. DoE under contract DE-FG02-89ER60858.

[1] Ratner, B.D., J. Molecular Recognition, 9 (1996) 617.
[2] Zhao, X.-M., Y. Xia, and G.M. Whitesides, J. Materials Chemistry 7 (1997) 1069
[3] S. Anders, et al. Rev. Sci. Inst. 70 (1999) 3973.

BI-WeP13 X-ray PhotoEmission Electron Microscopy of Polymeric Thin Films, ***A.P. Hitchcock***, *C. Morin,* McMaster University, Canada, *S.G. Urquhart*, University of Saskatchewan, Canada

Patterned thin polymer films are of increasing importance in biomaterials, displays, electronic materials, etc. High spatial resolution, high chemical sensitivity analytical microscopy techniques are needed to optimize these systems. We are using X-ray Photoemission Electron Microscopy (X-PEEM) for chemical imaging of phase segregated polymer blends, patterned biomaterials for non-fouling and bio-passivatable applications, and interactions of test proteins with these surfaces. Optimization of X-PEEM has been optimized for insulating, radiation sensitive organic thin films. Polystyrene (PS) and poly(methyl methacrylate) (PMMA) are completely immiscible and thus PS/PMMA blends may be a suitable model for patterned biomaterial-protein interactions. Spun cast thin film blends of mono-disperse high (1 Mdalton) and low (100 Kdalton) PS and PMMA with bulk compositions from 66/33 w/w up to 10/90 w/w PS/PMMA have been studied by X-PEEM and atomic force microscopy (AFM).[1] C 1s X-PEEM shows that there is significant enrichment of PS at the surface relative to the bulk and that the PMMA-rich domains contain PS. AFM shows the latter is a consequence of incomplete phase segregation, which results in a bimodal distribution of PS domain sizes, with the PS signal in PMMA domains arising from very small PS domains at the surface. This contribution will report on alternative techniques to prepare fully surface segregated PS-PMMA blends, and the outcome of protein attachment studies to these surfaces. X-ray microscopy carried out at the Advanced Light Source (supported by DoE under contract DE-AC03-76SF00098) and the Synchrotron Radiation Centre (supported by NSF under award DMR-0084402). Research supported financially by NSERC (Canada) and the Canada Research Chair Program. We thank the PEEM-2 staff (A. Scholl, A. Doran) for assistance in these studies.

[1] C. Morin et al., J. Electron Spectroscopy 121 (2001) 203.

BI-WeP14 NEXAFS Characterization of Poly (Amino Acids) at the Carbon, Nitrogen and Oxygen Edges, ***N.T. Samuel***, University of Washington, *D.A. Fischer,* National Institute of Standards and Technology, *D.G. Castner*, University of Washington

Near edge X-ray absorption fine structure (NEXAFS) spectroscopy has established itself as a powerful tool to characterize small molecules at interfaces. Recent developments in instrumentation have made it possible to image polymers and other biological molecules at very high spatial resolution and chemical specificity. In addition, NEXAFS offers the possibility to probe orientation and order in biological molecules at interfaces. Poly(amino acids) represent an important system of model compounds, since amino acids are the building blocks of proteins and peptides. Previous NEXAFS studies were done at the carbon and oxygen edges of individual and di amino acids. However, nitrogen is present in the backbone and some side chains of amino acids. In the present study, sixteen poly-amino acids were spin-cast or deposited as thick films onto silicon substrates. The samples were characterized by x-ray photoelectron spectroscopy (XPS) to ensure that a uniform film was obtained and no contaminants were present. The carbon edge spectra of these samples agrees well with the earlier work on amino acids. The nitrogen edge spectra of the poly(amino acids) exhibits three characteristic peaks, one due to the amide π^* resonance and the other two due to C-N σ^* resonances. Also, a pre-edge feature was observed that was associated with x-ray beam induced sample degradation. Overall, these poly-amino acids captured the major resonances in peptide and protein NEXAFS spectra. The different information content of NEXAFS and XPS will also be highlighted. These results coupled with previous results from our group indicate that NEXAFS can be used extract information about the orientation and order of biological molecules at interfaces.

BI-WeP15 Nanoporous Aluminum Oxide as Support Material for Enzyme Biosensors, ***A. Heilmann***, *N. Teuscher,* Fraunhofer-Institute for Mechanics of Materials, Germany, *D. Janasek, U. Spohn,* Martin-Luther-University, Germany

Enzyme sensors are of growing interest as detection tool in various clinical and food analysis. Up to now, limited operational stability of enzyme is even yet the main hindrance to their wider application to solve analytical problems. In the paper, we describe a novel method to create biosensors with good long-time stability by using nanoporous alumina oxide with well-defined pore sturcture as host material for enzyme immobilisation. The nanoporous aluminum oxide was made by anodic oxidation od alumnium in polyprotic acids. Free-standig membranes were realized by lifting the membrane film from the metal substrate. In this free-standig membranes, different enzymes were immobilized. e.g. pyruvate oxidase (PyrOD) from lactobacillus plantarum was enclosed in poly(carbamoylsulphonate) hydrogel and sucked into the porous structure before polymerization. In the paper, calibration curves and long-time stability of varous enzyme sensors were disccused, also by consideration the nanostructure of the filled alumina pores studied by scanning electron microscopy.

BI-WeP16 Formation of Biotin-reactive Silane Surfaces on SiO_2 for Specific Immobilization of Biomolecules, ***H.H.J. Persson***, *L.A. Ruiz-Taylor, D.A. Quincy, S. Follonier, J.K.C. Huang, T.L. Martin, A. Acharya, G. Kilcher, K. Belghiti, H.B. Lu, R.L. Cicero, P. Kernen, P. Wagner*, Zyomyx, Inc.

To provide robust and versatile surfaces for protein biochip applications, we have synthesized triethoxy silanes with thiol-reactive groups and report here on the formation of thin films on silicon oxide surfaces. Several surface analytical techniques such as contact angle, XPS, and ellipsometer have been applied to characterize such silane layers. Emphasis was given to layer thickness, wettability, molecular organization, homogeneity and reactivity. Effects of substrate pre-treatment, silanization conditions as well as post-silanization procedures have been evaluated. We used thiol reactive silane layers as reactive intermediates to produce reactive surfaces presenting biotin by in-situ coupling of PEGylated thiol-biotin crosslinkers. Streptavidin has been shown to bind to the surface specifically. Surface coverages, homogeneity, and reactivity of the streptavidin layer were evaluated mainly by radiometry.

BI-WeP17 Alkylsiloxane Self-assembled Monolayers on Titanium, *R.A. Brizzolara,* ***R.M. Lennen***, Naval Surface Warfare Center

Preparations of self-assembled monolayers (SAMs) of alkyltrichlorosilanes on silicon wafers or glass are well documented in the literature. On the other hand, little work has been done regarding SAM formation on other hydroxylated metal surfaces and little is known about the nature of the SAM. We have investigated the adsorption of different chain lengths of alkyltrichlorosilanes with different terminal functional groups on hydroxylated titanium as a function of reaction conditions using x-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and contact angle measurements. Angle-resolved XPS data was used for qualitative comparison of the vertical composition of the coatings, and the C1s/CKVV peak intensity ratio[1] enabled the calculation of comparative average layer thicknesses of the coatings. Reaction conditions included relative humidities of less than 1% and greater than 30%, with and without oven cure, and varying immersion times of the substrate in the adsorbate solution. In the future, these self-assembled monolayers will be used to investigate biofilm adhesion as a function of critical surface tension. This will lead to the development of ultra-thin antifouling coatings for shipboard titanium heat exchanger tubes with seawater intake. This work was funded by the NSWC Carderock Division In-House Laboratory Independent Research program.

[1] Brizzolara, R. A.; Beard, B. C.; Surf. Interface Anal., 27, 716 (1999).

BI-WeP18 A Novel Approach for the Detection of Antibiotics using Mixed SAMs of Thiols on Gold, ***K. Bonroy***, *F. Frederix,* IMEC, Belgium, *P. Cliquet,* University of Ghent, Belgium, *W. Laureyn, A. Campitelli, G. Borghs,* IMEC, Belgium, *E. Cox,* University of Ghent, Belgium, *P. Declerck*, KULeuven, Belgium

In numerous applications in the clinical, environmental and toxicological field, there is an increasing need for the detection of low concentrations of different biochemical parameters with low molecular weight. For example, the detection of antibiotic residues in milk is important because of the potential toxic and allergic effect for humans. Immunosensors have a huge potential as a fast and reliable system for the detection of such low molecular weight analytes. Our research is not only focussing on the transducer part of a biosensor, but also on the biological recognition layer. This biological recognition layer mainly determines the specificity, stability, reproducibility and durability of the biosensor. For the detection of small molecules, different assay principles are possible. In the current study

an indirect competitive assay was used. Therefore antibiotics were immobilised on the sensor surface and the optimal concentration of a monoclonal antibody (directed against b-lactam antibiotics), allowing quantification of antibiotics, was established.[1] For this competitive method, a reproducible and tuneable immobilisation of the antibiotics on the sensor-surface is indispensable. In order to realise such an immunosensor we coupled the antibiotics covalently to mixed Self-Assembled Monolayers (SAMs) of thiols deposited on gold. These mixed SAMs consist of two different thiols: one to bind the antibiotics and another thiol component to avoid non-specific adsorption 2. The binding of the antibiotics on mixed SAMs was characterised with cyclic voltammetry and GA-FTIR and the competitive immunoassay was evaluated using Surface Plasmon Resonance. In conclusion, we have developed a competitive immunoassay for the detection of antibiotics using a biosensor interface based on mixed SAMs of thiols.

[1] Cliquet P. et al. (2001).Journal Agricultural Food Chemistry, 49, 3349-3355
[2] Frederix F. et al. (2001).European Conference Organised Films, P11.03.

BI-WeP19 Poly(ethylene oxide)-Terminated Monolayer Formed at Solid/Vapor Interface, ***A. Hozumi***, *Y. Yokogawa, T. Kameyama*, National Institute of Advanced Industrial Science and Technology, Japan

Poly(ethylene oxide) (PEO) has been widely applied to the fabrication of protein or cell repellent surfaces in biotechnical and biomedical applications. Although extensive research on PEO-coating techniques has been reported in the literature, there have been few reports on preparing PEO-terminated monolayers from the vapor phase. Here, we report on the formation of a PEO-terminated monolayer on SiO_2/Si sample substrates through a chemical vapor deposition (CVD) method. Si substrates covered with na tive oxide (SiO_2/Si) were first cleaned by UV/ozone treatment. The cleaned samples were then exposed to vapor of organosilane, that is, 2-[methoxy (polyethyleneoxy) propyl] trimethoxysilane (MPEOPS) for 1~7 hours at 150 °C. We have investigated in detail chemical and electokinetic properties of this PEO-terminated monolayer. The SiO_2/Si surface after CVD became relatively hydrophobic showing a water-contact angle of ca. 67±2. Thickness of the MPEOPS-monolayer was ca. 0.8±0.1 nm as estimated by ellipsomrtry. As confirmed by AFM, the surface was very smooth and homogeneous with almost identical to that of the SiO_2/Si substrate. Zeta-potentials of the MPEOPS-monolayer covered SiO_2/Si substrates were measu red as a fun ction of pH by means of an electrophoretic light scattering spectrophotometer. Isoelectric point of the MPEOPS-monolayer covered surface was observed at around pH 5 which was higher than that of SiO_2/Si (~pH 2.0). These results are att r ibutable to a reduction in the density of surface silanol (Si-OH) groups on the SiO_2/Si substrate. Si-OH groups were consumed due to the formation of siloxane bondings with the MPEOPS. Furthermore, we demonstrated micropatterning of this MPEOPS-mo no layer based o n the photolithography using an excimer lamp radiating vacuum ultra violet light of 172 nm in wavelength.

BI-WeP21 Study of Bone Repairing Employing a Ricinus Camunis-based Biopolymer Additioned with Ascorbic Acid and Epidermal Growth Factor in a Rat Tibia Model[1], *C. Mendoza-Barrera*, UPIITA-IPN and Cinvestav-IPN, Mexico, ***M. Melendez-Lira***, *V. Altuzar, S.A. Tomas*, Cinvestav-IPN, Mexico

There is a huge demand of graft material to accelerate the bone healing process of lesions experienced after surgical interventions related with bony tumors or traumatic experiences. Autografts, allografts and biomaterials are sources of graft materials but it would be highly desirable to have materials without the limitations of the first two aforementioned. We discuss the effect of a Ricinus Camunis-based biopolymer mixed with Ascorbic Acid (AA)and/or Epidermal Growth Factor (EGF) on the bone repairing employing a rat tibia model. The bone healing process was monitored through the interface bone-graft material. No implant rejection or inflammatory reaction was observed during a 8 weeks period in our in vivo studies. The evolution of the osteogenesis in the lesion area was followed employing scanning electron microscopy (SEM), energy dispersive x-ray analysis (EDX), Photoacoustic Spectroscopy (PAS) and X-ray diffraction (XRD). Our study clearly indicates that the combined use of the Ricinus Camunis-based biopolymer, AA and EGF improves the remodeling characteristics of the biomaterial. XRD allow us to identify the structural characteristics of the biopolymer and its evolution during the bone healing process. Bone resorption was monitored by EDX studying the Ca/P ratio as function of time. PAS presents features that could be correlated with cellular activity during the bone mineralization process. All the results showed a good correlation and allowed us to obtain information to improve the composition of the biomaterial.

[1]This work was partially supported by CONACYT-Mexico.

Biomaterials
Room: C-201 - Session BI-WeA

Polyelectrolyte Surfaces/Cell-Surface Interactions
Moderator: A. Chilkoti, Duke University

2:00pm **BI-WeA1 Polyelectrolyte Multilayers: Design of Biofunctional Surfaces**, ***G. Decher***, Université Louis Pasteur, France **INVITED**

Layer-by-layer (LbL) assembly@super 1@ is an easy to use method for the fabrication of multi-composite films and has kindled widespread interest in such nanohybrids.@super 1-@super 7@. Electrostatic interactions between anionic and cationic compounds (synthetic or natural polyelectrolytes, DNA or proteins) offer four major advantages: (1) layer-by-layer construction due to surface charge reversal in each layer (2) restriction to single layers due to repulsion between last layer and excess material (3) low steric demand for interaction between oppositely charged ions (4) deposition on almost any solvent accessible surface. As an introduction to the LbL-technique, the guiding principles of multilayer assembly will be presented and details of the film structure will be discussed. Since the technique allows to interface a wide variety of materials with predefined spatial arrangement, it has successfully been introduced to both materials science and applied bio-sciences. At this meeting we will focus on work relevant for surfaces in contact with biological materials or environments. This will include work on films composed of natural or semi-synthetic polyions such as charged polypeptides or polysaccharides some of which has been carried out in close collaboration with the groups of P. Schaaf (ICS) and J.-C. Voegel (INSERM U 424).

[1] Decher, G., in: Comprehensive Supramolecular Chemistry, Vol. 9, (Sauvage, J.-P. and Hosseini, M. W., Eds.), Pergamon Press: Oxford, 1996; 507-528.
[2] Knoll, W., Curr. Opinion in Coll. & Interface Sci. 1996, 1, 137-143.
[3] Decher, G., Science 1997, 277, 1232-1237.
[4] Laschewsky, A., Europ. Chem. Chronicle 1997, 2, 13-24.
[5] Decher, G. et al., Curr. Opinion Coll. & Interf. Sci. 1998, 3, 32-39.
[6] Bertrand, P. et al., Macromol. Rapid. Commun. 2000, 21, 319-348.
[7] Hammond, P. T., Curr. Opinion Coll. & Interf. Sci. 2000, 4, 430-442.

2:40pm **BI-WeA3 Poly-(L-Glutamic Acid)/Poly-(L-lysine) Multilayers used as Biomaterial Coating**, ***P. Schaaf***, Institut Charles Sadron (CNRS), France, *J.C. Voegel, C. Picart, Ph. Lavalle*, Unite INSERM U424, France, *F. Boulmedais*, Institut Charles Sadron (CNRS), France

Polyelectrolyte multilayers constitute an easy tool for multi-functionalizing biomaterial surfaces. In this paper we will discuss biological and physico-chemical properties of poly-(L-glutamic acid)/poly-(L-lysine) (PGA/PLL) multilayers. We first discuss the response of cells in contact with (PGA/PLL) multilayers functionalized by embedding active peptides within the films. We then present results relative to the cellular response when the film is functionalized by embedding proteins in the architecture. In both cases we get a cellular response even when the active molecules are embedded under up the 20 (PGA/PLL) bilayers. We discuss also possible cell recognition mechanisms of the active molecules. But the system (PGA/PLL) is also interesting because it leads to films with internal secondary structures such as beta-sheets or alpha-helices. The relation between the secondary structure of the film and the secondary structure of (PLL/PGA) complexes in solution will also be presented.

3:00pm **BI-WeA4 Cellular Interactions with Multilayered Polyelectrolyte Films**, *C. Picart*, Université Louis Pasteur, France, *Ph. Lavalle*, INSERM Unite 424, France, *L. Richert, D. Vautier*, Université Louis Pasteur, France, *P. Schaaf*, CNRS, France, ***J.C. Voegel***, INSERM Unite 424, France

The short time interactions of chondrosarcomas cells with polyelectrolyte multilayered architectures built up by the alternated deposition of poly(L-lysine) (PLL) and poly(L-glutamic acid) (PGA) were estimated in the presence and the absence of serum. Film constructions with and without protein adsorption were first characterized by means of optical waveguide lightmode spectroscopy, quartz cristal microbalance and zeta potential determinations. In the presence of a serum containing medium, the detachment forces measured by the micropipette technique were about eight times smaller for PGA than for PLL ending films. For these later ones, the adhesion forces decreased also when the films increased in thickness. In a serum-free medium the differences between the negative and positive ending films become larger : adhesion forces on PLL-ending films were by 40 % to 100 % higher, whereas no cells adhered on PGA terminating films. Also, PGA ending films were found to prevent the adsorption of serum proteins whereas large protein amounts adsorbed always on PLL ending films. These data suggest that cell interactions with polyelectrolyte films can be tuned by the type of the outermost layer, by the presence of proteins, and the number of deposited layers.

3:20pm **BI-WeA5 Entrapment of Phosphate Ester Hydrolyzing Enzymes in Polyelectrolyte Multilayers Deposited on Glass Beads and Extended Retention of Their Enzymatic Activity**, ***A. Singh***, *Y. Lee, I. Stanish*, Naval Research Laboratory, *T.C. Cheng*, Edgewood Research Development & Engineering Center, APG, MD

Recent advances in multilayer technology involving layer by layer technique indicate its utility in solving complex problems of multidisciplinary nature. We have explored layer-by-layer technology for entrapping enzymes organophosphorus hydrolase and Organophosphorus acid anhydrolase in polyelectrolyte multilayers with a goal to sustain their biological activity for a long period of time under an environment, where native enzymes turned inactive. Thus, phosphate ester-hydrolyzing enzymes were immobilized in multilayers coated on glass beads (30-50 μm). Coatings on Glass beads consist of 3 alternating layers of branched poly (ethyleneimine) (BPEI) and polystyrene sulfonate (PSS) as precursor layers, followed by five alternative layers of BPEI and OPH. Immobilized enzymes were tested for their enzymatic activity and stability at different temperature and under different humidity conditions and found active. Surprisingly, in 15 percent relative humidity environment an enhancement in enzyme activity was observed. Stability of multilayers incorporating enzymes was further improved by laying additional poly (acrylic acid) (PAA) layer on top of the multilayer assemblies and endcapping the enzyme-PAA layer with monomers, such as trimethylsilyl propylethylenediamine (TMSPED), and vinyl benzyl solketol (VBS). TMSPED end-capped OPH enzyme performed better than VBS coated multilayers and was further evaluated through salt stress test (involving 1 M aq. NaCl). An improved performance of end-capped OPH glass beads was demonstrated than their uncapped counterpart. Efforts on the synthesis of novel support beads will also be presented.

3:40pm **BI-WeA6 In-situ Measurements of Polyelectrolyte Multilayer Build-up using Ellipsometry and QCM-D : 9**, ***T.J. Thurell***, *U. Elofsson*, YKI, Sweden

Polyelectrolyte multilayers are easily constructed by alternately exposing a charged surface to positively and negatively charged polymers. The aim of this study was to create a biocompatible surface for use in implant technology. The charged poly-amino-a cids PGA (Poly (L)Glutamic Acid) and PLL (Poly (L)Lysine) were coated on an initial layer of PEI (Polyethylimin) on both silica and titanium surfaces. Multilayer build-up was monitored in-situ using both ellipsometry and QCM-D. The polyelectrolytes adsor b ed firmly, with insignificant desorption upon rinsing, on both substrates used. In the ellipsometer up to 12 layer pairs were easily built while monitored in-situ. Comparing adsorbed amounts obtained from the ellipsometer, with those calculated from QC M-D measurements, one can see that these polymer-multilayer-films are highly hydrated (app. 70% water content). In the QCM-D measurements, an almost linear mass increase/layer are obtained from the second layer pair and up, whereas linear mass increase/l a y er is not achieved until the eighth layer pair in the ellipsometer. The observations indicate that the polymer film become denser with each added layer. This is also reflected in the increasing refractive index, which eventually level out at 1.457 af t er abo ut 10 layer pairs. The large fluctuations in refractive index and thickness found initially suggests that the polyelectrolytes in the two first layer pairs are unevenly adsorbed with gaps and holes where water may get trapped. One possible explanat io n for the variations in film density would be that the gaps and holes will eventually fill up resulting in constant film density and linear mass growth. This work is a part of the SIMI project (Surface Improvement of Metal Implants GRD1-2000-26823) funded by the European Commission.

4:00pm **BI-WeA7 Nanoscale Surface Properties of Microbial Cells**, ***Y.F. Dufrene***, Universita Catholique de Louvain, Belgium **INVITED**

Biological events such as microbial adhesion, microbial aggregation and molecular recognition play a pivotal role in the natural environment, in medicine and in biotechnological processes. Understanding the molecular bases of these phenomena requires knowledge of the structural and physical properties of microbial cell surfaces. With atomic force microscopy (AFM), it is now possible to explore the surface of single cells with nanometer lateral resolution and under physiological conditions. AFM can be used for visualizing surface ultrastructure (crystalline arrays, appendages), for following physiological changes (germination, growth) and for monitoring the effect of external agents (antibiotics, metals). These studies open the door to new applications in biotechnology and biomedicine, such as the

rapid detection of microorganisms and the rationale design of drugs. AFM is actually much more than a microscope in that it also enables physical properties to be probed quantitatively: (i) surface hydrophobicity and electrical properties can be mapped using probes functionalized with defined functional groups (CH3, OH, COOH); (ii) surface softness can be measured by pressing the probe onto the cell surface; (iii) the elasticity of surface macromolecules, such as polysaccharides, can be addressed by means of force spectroscopy. These measurements have a great potential for elucidating the structure-function relationships of microbial surfaces (molecular recognition, conformational changes, surface interactions). In this contribution, I will discuss recent data obtained on fungal spores, yeasts and bacteria to highlight these unique capabilities.

5:00pm **BI-WeA10 Development of a Fluorescent Based Assay for Quantifying Ligand Surface Density on IPN-Modified PS for High Throughput Applications**, ***G.M. Harbers***, Northwestern University, *T.A. Barber*, University of California, Berkeley and University of California, San Francisco, *K.E. Healy*, University of California, Berkeley, *S.L. Golledge, D.G. Castner*, University of Washington

Biomimetic surface engineering exploits the power of specific ligand-receptor engagement to control cell-biomaterial interactions independent of bulk material characteristics. Accurate characterization of ligand surface density (Γ) is crucial for interpreting cellular response to these engineered surfaces. Currently, low throughput techniques including ellipsometry, SPR, and radiolabeling are employed to make these measurements. Lack of high throughput alternatives provided the motivation for the development of a fluorescence microplate reader based assay to measure Γ on a modular biomimetic surface developed to rapidly screen the adhesive potential of bioactive peptides. Poly(acrylamide-co-ethylene glycol/acrylic acid) interpenetrating polymer networks [p(AAm-co-EG-/AAc) IPNs] were grafted on to 96-well polystyrene (PS) plates. Fluorescently labeled peptides were subsequently coupled to the IPN using different input concentrations (0.01-100 μM) to modulate Γ. Surface characterization (contact angle goniometry and XPS) and cell-surface interactions were consistent with the results on previously developed IPN modified metal oxide surfaces. Reproducible control of Γ was observed over four orders of magnitude (~ 0.1-100 pmol/cm^2). Furthermore, competitive binding experiments using labeled and unlabeled peptides facilitated the determination of the equilibrium dissociation constants (K_d) of the various peptides. Although this technique may not be as sensitive as the others mentioned above, it allows for the characterization and rapid development of well defined biomimetic surfaces for high throughput applications.

Homeland Security
Room: C-209 - Session HS+SS+BI-WeA

Chemical and Biological Detection
Moderator: J.N. Russell, Jr., Naval Research Laboratory

2:00pm **HS+SS+BI-WeA1 Photonic Crystals Derived from Nanocrystalline Porous Si: Applications in Detection of Chemical Warfare Agents, Explosives, Pollutants, and Biochemicals**, ***M.J. Sailor***, University of California, San Diego **INVITED**

The optical properties of nanostructured porous silicon films are exploited for a variety of sensor applications. With appropriate modification of the electrochemical preparation conditions, multilayered structures can be generated that behave as photonic crystals. These structures can be encoded and used as remote sensors for chemicals. For example, small particles of nanoencoded microporous Si are used to detect chemicals by measurement of the intensity of reflected light from a remote laser probe. The particles contain a periodic porous nanostructure that defines the code. The periodic structure forms a Rugate reflector which displays sharp maxima in the optical reflectivity spectrum at wavelengths that are controlled by the etch parameters. The intensity and wavelength of reflected light is determined in part by the refractive index of the porous nanostructure, which can be modified by adsorption of vapors within the porous matrix. Using a 10 mW laser as an optical probe and telescope collection optics, detection of ethanol, acetone and toluene vapors has been achieved at a distance of 20 m. Control experiments using water vapor at comparable partial pressures show very little response, demonstrating selectivity towards the hydrocarbon analytes. Examples of irreversible detection and reversible sensing modes for explosives, nerve warfare agents, and various biochemicals will also be discussed. A catalyst can be incorporated into the nanomaterials to provide specificity for nerve warfare agents. For example, rapid detection of a fluorophosphonate is achieved by catalytic decomposition of the agent to HF and subsequent detection of the HF in the porous silicon interferometer. The catalyst system can be integrated on the silicon chip and consists of a TMEDA[Cu(II)] catalyst (TMEDA = tetramethylethylenediamine) encapsulated in cetyltrimethylammonium bromide (CTABr) micelles. An operational battery-powered unit has been constructed and tested on the live nerve warfare agent Sarin. These devices are all compatible with conventional Si microfabrication technologies.

2:40pm **HS+SS+BI-WeA3 Magnetic Labeling and Microarray Detection of Biomolecules**, ***L.J. Whitman***, Naval Research Laboratory **INVITED**

NRL is developing two novel biosensor systems using magnetic microbeads to probe for target biomolecules specifically bound to receptor-patterned surfaces, with an initial focus on detecting biological warfare agents.[1,2] The microbeads serve both as reporter labels and as force transducers to allow "force discrimination" - a technique developed at NRL that greatly reduces the background signal-enabling the identification of single biomolecular ligand-receptor interactions with high sensitivity and specificity. Assays using magnetic labeling and force discrimination have been developed for a variety of bacteria, viruses, and protein toxins (immuno-sandwich assays), and for oligonucleotide microarrays (hybridization assays). How the assays are incorporated into a practical sensor system depends on how the specifically bound beads are detected. We are currently perfecting two detection approaches, an optical system that images beads captured on a patterned nanoporous membrane, and a chip-based sensor system that directly detects beads using an array of giant magnetoresistive (GMR) magnetic field microsensors. The optical system has achieved sensitivities of 10 pg/ml for proteins, $10^{2.5}$ cfu/ml for bacteria, and 10^3 pfu/ml for viruses. Using a single GMR sensor, we have successfully detected 1 fM of DNA in a 30 μL sample with only 15 min of hybridization. I will discuss how the interplay between surface chemistry, sensor design, and microfluidics determines the overall performance of our biosensor systems. Supported by ONR, the DoD JSTPCBD, and DARPA.

[1]Lee et al., Anal. Biochem. 287, 261 (2000).
[2]M. M. Miller et al., J. Mag. and Mag. Mat. 225, 138 (2001).

3:20pm **HS+SS+BI-WeA5 Optical Microarrays for Chemical and Biological Detection**, ***D.R. Walt***, Tufts University **INVITED**

We have used coherent imaging fibers to make fiber-optic chemical sensors. Sensors can be made with spatially-discrete sensing sites for multianalyte determinations. We are investigating the limits of our ability to create high-density sensing arrays containing thousands of microsensors and nanosensors. Micrometer- and nanometer-sized sensors have been fabricated by etching the cores of the optical imaging fiber to create wells and loading them with micro and nanospheres. Such arrays can be employed for making genosensors for bio-agent detection. We have also created optical sensors based on principles derived from the olfactory system. A cross-reactive array of sensors is created such that specificity is distributed across the array's entire reactivity pattern rather than contained in a single recognition element. The ability to use such information-rich assemblies for broad-based chemical sensing will be discussed.

4:20pm **HS+SS+BI-WeA8 Real-time Detection of TNT Using Microcantilevers with Microcyclic Cavitand Coatings**[1], ***N.V. Lavrik***, *T. Thundat, G. Muralidharan, P.G. Datskos*, Oak Ridge National Laboratory

Real-time detection of nitroaromatic aromatic explosive compounds in various environments is a highly significant task in forensics, anti-terrorist activities and global de-mining projects. In particular, ability to detect trace levels of trinitrotoluene (TNT) in air and soil could greatly reduce continued fatalities from land mines among civilians and be a measure in tracking and locating explosive materials. In our work, we address this challenge of detecting TNT vapors in gaseous environment by using an innovative, highly sensitive microcantilever transducer combined with a chemically sensitive molecular coating based on the macrocyclic cavitand of a calixarene family. We measured responses to vapors of TNT and its analogs, 0-mononitrotoluene and 2,4-dinitrotoluene vapors in the range of temperatures of 298 K to 318 K. Our results were used in order to estimate the limits of detection (LODs) for these compounds and optimize the temperature regime of the designed detection system. In the case of TNT, the steady state responses were large, however, the response kinetics was significantly elongated, which is consistent with an analyte depletion model. As compared to more traditional surface acoustic wave sensors with a proven potential for detection of TNT, our approaches offer a simpler, low-cost alternative without sacrificing the performance. The reported results together with these advantages of microcantilever based gas detectors clearly indicate a viable technological approach to mass produced detectors of explosive materials.

[1] This work was supported by the U.S. Department of Energy and Micro Sensor Technologies, Inc. Oak Ridge National Laboratory is operated for the U.S. Department of Energy by UT-Battelle under contract DE-AC05-96OR22464.

4:40pm **HS+SS+BI-WeA9 A New Nanoscale Platform for Gas Sensor Applications**, *A. Kolmakov, Y. Zhang, G. Cheng,* ***M. Moskovits***, University of California Santa Barbara

The application of metal and semiconductor nanowires as solid state gas sensors has been an area of tremendous promise currently limited by challenges related to nanowire growth and device fabrication. We present an approach for fabricating individual and arrays of nanowires of a variety of metals and metal oxides with tunable, uniform diameters and length in the range of 10-100 nm and 5-200 micrometers, respectively, configured for gas sensing application. The materials successfully employed include Pd, Ag, Cu, Pb, PbO, CuO and SnO_2. Arrays of nanowires were fabricated in hexagonal close-packed nanochannel alumina templates. Electrodes deposited on the surfaces of these nanostructures provides electrical contacts which with the incorporated heaters determines the device architecture. Based on this method we explored the electronic and structural properties of Pd and SnO_2 nanowires using HRTEM, XPS and Auger spectroscopy. Chemical reactivity and gas sensitivity toward hydrogen and carbon monoxide of individual and assemblies of ca 10^9 Pd and SnO_2 nanowires were assessed using conductivity measurements and TPD analysis. This approach constitutes a novel platform for micro- and nanosensor application.

5:00pm **HS+SS+BI-WeA10 Metal Phthalocyanine Thin Films as Gas Sensors**, ***L. Lozzi***, *S. Santucci*, INFM and University of L'Aquila, Italy, *C. Cantalini*, University of L'Aquila, Italy

Metal Phthalocyanine (MPc) thin films have shown interesting properties as gas sensor, in particular for NO2. The wide variety of different available molecules, changing both the central atom and/or the chemical structure of the outer benzene rings, allows a fine modulation of the film sensing properties. In this work we will present our result on the interaction between oxidating gases (O2 and NO2) and different MPc films. We have deposited thin films (about 50 nm thick) of Copper Phthalocyanine (CuPc) and Exadecafluoro-copper-phthalocyanine (F16CuPc) onto Si3N4 substrates, for the spectroscopic characterizations, and onto Pt interdigital circuits, for the gas sensing tests. These films have been analysed both as deposited and after different thermal annealing. The electrical sensing analyses have shown a sizeable decrease of the film resistivity during the film exposure to NO2, even at very low concentration (up to 100 ppb). We have studied the electronic structure by means of the X-ray and ultraviolet photoemission spectroscopies (XPS-UPS) after the exposure to NO2 and O2 both at room and at higher temperature, in order to investigate the surface reactivity of these samples and in particular the preferential adsorption sites.

Plasma Science
Room: C-103 - Session PS+BI-WeA

Plasma Processing for Biocompatible Surfaces
Moderator: H.J. Griesser, University of South Australia

2:00pm **PS+BI-WeA1 Application of Plasma in Tissue Engineering**, ***R.D. Short***, *D.B. Haddow, S. MacNeil, R.A. Dawson, D. Barton, S. Fraser*, University of Sheffield, UK **INVITED**

A novel device which comprises an acrylic acid plasma polymerized carrier substrate, which supports the attachment and release of human keratinocytes, has been used to successfully treat non-healing (chronic) skin wounds. In proof of concept studies, weekly delivery of keratinocytes, initially obtained from a small 2x1cm biopsy and expanded up many hundred fold, have promoted healing in diabetic foot ulcers and other indications. In this paper we explain the rationale behind this therapy and review the results (to date) from the treatment of the first seven patients. Although the "mode of action" of the device is still to be fully understood, the role the plasma polymer plays in promoting cell attachment and detachment is key to the success of the device. The physical and chemical nature of the plasma polymer has been explored in some detail, and to a first approximation, we are able to describe the features of the plasma polymer that promote cell attachment and speculate on why cells transfer to an in vitro human dermal wound bed model. By means of a multi-technique approach (mass spectrometry,quartz crystal microbalance, ion flux probe, x-ray photoelectron spectroscopy, secondary ion mass spectrometry) we have begun to unravel the processes by which the acrylic acid plasma polymer grows. A case is made for better understanding of plasma polymer growth mechanisms, rather than treating the plasma polymerization process as a "black box" that produces materials with desired properties.

2:40pm **PS+BI-WeA3 Can Plasma Polymerised Surfaces Promote the Co-culture of Human Dermal Fibroblasts and Human Epidermal Keratinocytes in the Tissue Engineering of Skin?**, ***M.C. Higham***, *S. MacNeil, R.D. Short*, University of Sheffield, UK

Within the field of tissue engineering there is a need to develop new approaches to achieve effective wound closure in patients with extensive skin loss or chronic ulcers. Plasma polymers are synthetic surfaces capable of influencing and controlling cell physiology either directly or through an adsorbed protein layer. This project exploits the well-known interdependency of epithelial keratinocytes and stromal fibroblasts in conjunction with plasma surface technology. The aim of my project is to produce a chemically defined surface, which with the aid of a feeder layer of lethally irradiated dermal fibroblasts will improve the performance of the keratinocyte cell. Unable to divide yet remain physiologically active, irradiated fibroblasts aid keratinocyte attachment and proliferation from which sub-confluent cells can be transferred to wound bed models. Plasma co-polymers of acrylic acid/octa-1,7-diene have been prepared and characterised using X-ray photon spectroscopy (XPS). The fibroblasts and keratinocytes were cultured on plasma polymer coated 24 well plates. Cell attachment and proliferation were assessed using MTT-ESTA and DNA assays. The performance of both cell types on the plasma polymer surfaces was compared to Tissue Culture Plastic (TCPS) and Collagen I, plus a negative control of a pure hydrocarbon layer. A pure acrylic acid surface, fabricated at a power of 10W and containing 9% carboxylate group was found to promote both fibroblast and keratinocyte attachment and proliferation and permit the co-culture of keratinocytes with irradiated fibroblasts. The performance of this surface was comparable to collagen I, a well-established substratum for the attachment of keratinocytes. Current work is examining the potential of plasma polymer surfaces within the field of tissue engineering for transfer of keratinocytes onto an in vitro wound bed model and thereafter clinical trials.

3:00pm **PS+BI-WeA4 The Role of Reactive Neutral and Ionic Species in the Deposition of Organic Thin Films from an Isopropyl Alcohol and Argon Plasma**, ***D.C. Guerin***, National Research Council, Canada, *V.A. Shamamian*, Naval Research Laboratory

We present the measurements of neutral species in an argon/isopropyl alcohol (iPrOH) plasma, using appearance potential mass spectrometry. IPrOH is a potential precursor for the cost-effective plasma deposition of non-fouling surfaces. This work complements previous research on the ionic character of the plasma. It had been discovered that tuning the plasma pressure and power caused large variations in the dominant ionic reactions. The resulting changes in the chemical nature of the ionic flux were reflected in the functional character of the deposited films. A significant flux of neutral radicals was detected at the deposition surface at low plasma pressure. However, at higher pressures the plasma region was more remote and the neutral radicals were completely attenuated. The attenuation mechanism was determined to be reaction with the precursor. For example, the methyl radical abstracts hydrogen from iPrOH. Thus, as the pressure increases, the methyl radical flux evolves into a flux of methane. Mean free path (MFP) calculations for hydrogen abstraction agree with the experimental results. At low pressures, the reactive MFP is larger than the chamber geometry. At higher pressures, the reactive MFP is much smaller than the distance between the plasma and deposition surface. The ability of the reactive ions to diffuse from the remote plasma to the deposition surface is explained as being due to charge exchange limitations. The radical species generated have lower ionization energies than iPrOH or argon. Thus, the radical ions are energetically unable to react with the main species in the plasma. In contrast, the flux of ions with ionization energy greater than that of iPrOH, such as argon and methane, is highly attenuated at higher pressures. These results provide some context to competing claims as to the importance of neutrals and ions in deposition from molecular plasmas.

3:20pm **PS+BI-WeA5 Plasma Micropatterning for the Spatially Controlled Adsorption of Proteins**, ***J.D. Whittle***, *R.D. Short, D. Barton, A.G. Shard*, University of Sheffield, UK

Many biological interactions are surface mediated, for example protein adsorption and subsequent cell adhesion. In vitro it may be desirable in a number of applications to exert spatial control over these interactions. i.e. Limiting the attachment of cells to particular surface regions. We investigate the use of masks as a method of fabricating surfaces with patterned chemistry by plasma polymerisation, with feature sizes down to around 10µm. We utilise imaging secondary ion mass spectrometry (SIMS) and fluorescent light microscopy to visualise these chemical patterns. We also show how these chemical patterns affect the adsorption of proteins, not only in terms of the the amount of adsorbed protein, but also their conformation. A natural extension of depositing well-defined regions of chemistry (patterns) is to be able to fabricate regions of controlled chemical

change (gradients), the properties of which vary continuously along the length of the deposited feature without any sharp transitions. We show how plasma polymerisation may be used to deposit chemical gradient surfaces with chosen endpoints (for example, a gradient running from a hydroxyl though to an amine dominated surfaces), and profile (for example, linear, sigmoidal etc.) by careful manipulation of the plasma composition and deposition surface during the treatment. These gradient surfaces can be used to examine the affect of changing a particular surface parameter (for example, the surface concentration of amine functionalities) on protein adsorption.

3:40pm **PS+BI-WeA6 Chemical Surface Micropatterning by Plasma and VUV Photochemical Modification of Polymers for Controlled Cell Culture.**, ***N.A. Bullett***, *F.E. Truica-Marasescu, M.R. Wertheimer*, Ecole Polytechnique, Canada

The three dimensional nature of the biomolecular environment in contact with cells has an important influence on the initiation and control of cell processes such as adhesion, migration, growth, protein secretion and gene expression. Traditionally, cell culture uses homogeneous substrates with no control over the biochemical and topological features in the immediate vicinity of the cells. The shape of mammalian cells is determined by the interaction of cell contact receptors with other cells or extracellular matrix proteins. Regulation of the shape of cells may enhance the function and differentiation of the cells. Surface modification of polymeric materials by low-pressure plasma and VUV photochemical treatment provides a convenient route to the fabrication of well defined chemically functionalised surfaces. A variety of functional groups may be introduced into the polymer surface, including amine and hydroxyl. Using these techniques it is possible to engineer surfaces that have a wide variety of applications in biomaterials technology, such as cell and protein adhesive surfaces or non-fouling surfaces. Complex micropatterns of chemically different regions have been produced by the selective functionalisation of the polymer using photolithographically defined masks. By this method, chemically distinct regions are produced at the micrometer scale, with a third dimension being provided by nanoscale topographical features. This three dimensional environment, on the nano- or micrometer scale, provides a complex but controllable surface for the culture of many different cell types. Characterisation of the micropatterned surfaces has been performed by XPS, FTIR, imaging TOF-SIMS and fluorescence microscopy. The surfaces have subsequently been used to study the attachment and growth of various cell types, for example bone-derived cells with orthopaedic applications.

4:00pm **PS+BI-WeA7 Study of Adhesion Mechanism of Protein-based Hydrogel to Plasma Treated Polymer Surface**, ***O. Zabeida***, Ecole Polytechnique of Montreal, Canada, *M.-P. Faure*, Bioartificial Gel Technologies, Canada, *J.E. Klemberg-Sapieha, L. Martinu*, Ecole Polytechnique of Montreal, Canada

Biodegradable protein-based hydrogels (solid water solutions, SWS^{TM}) are a new class of biomaterials with great potential for use in numerous pharmaceutical and medical applications. Since they may contain up to 96% of water, some SWS are rather fragile and difficult to handle and manipulate. This problem can be solved by applying appropriate polymer backings; the latter one has to be surface treated in order to enhance the hydrogel's adhesion. We found that plasma modification of polymer backings can lead to a 20-fold increase of the adhesion force between the SWS and the polymer surface. In the present work we have applied a multitechnique surface analytical approach, including infrared spectroscopic ellipsometry, XPS, AFM, and TOF-SIMS, to investigate the adhesion mechanism of hydrogels to low pressure plasma-treated polymers (polypropylene, polyethylene terephthalate, and others). The surface chemical structure and morphology are correlated with the adhesion force of the SWS. The results suggest that introduction of amine groups plays a major role in the adhesion improvement, while the surface roughening, polymer chain scission and surface electric charge should also be considered.

4:20pm **PS+BI-WeA8 Permanent Hydrophilic Modification of Porous Membranes Using Low-Temperature Plasmas**, ***D.S. Wavhal***, *E.R. Fisher*, Colorado State University

We have explored the use of low-temperature plasmas to modify porous polymeric membranes with the goal of creating hydrophilic surface throughout the membrane structure. One motivation for this work is to decrease membrane fouling and to eliminate the need for wetting agents in a variety of applications. Porous polyethersulfone (PES) membranes were modified by CO_2 plasma treatment and Ar-plasma treatment followed by grafting of hydrophilic monomers (acrylic acid and acrylamide), in the vapor phase. Plasma treatment and plasma induced grafting rendered a complete hydrophilicity to the entire PES membrane cross section. The hydrophilicity of the membranes treated with only the Ar-plasma is not, however, permanent. In contrast, the PES membranes treated with CO_2 plasma and the grafted membranes are found to be permanently hydrophilic (for a minimum of six months). Chemical changes to the modified PES membranes were determined with FTIR and XPS measurements. Furthermore, water bubble point measurements and electron microscopy results reveal that pore sizes of the modified membranes are slightly affected. The pore sizes of the grafted membranes at higher grafting yield are slightly decreased. Due to incorporation of polar functionalities, the glass transition temperature (T_g) of modified membranes also increases. A moderate change in tensile strength of the modified membranes was observed. Most importantly, the surface of the modified membrane are less susceptible to absorbtion by bovine serum albumin (BSA) proteins and give greater flux recoveries. This suggests that the protein fouling layer is reversible because of hydrophilic nature of the modified membranes.

4:40pm **PS+BI-WeA9 Acrylic Acid Films Deposition by RF PACVD: Relation between Monomer Fragmentation and Surface Properties**, *P. Rossini, G. Ceccone*, European Commission, Joint Research Centre, Italy, *K. Jandt*, University Jena, Italy, ***F. Rossi***, European Commission, Joint Research Centre, Italy

The present study deals with the deposition of acrylic acid thin films by radio frequency plasma assisted chemical vapour deposition. The experiments have been carried out in a cylindrical capacitively coupled plasma reactor at different electrical powers (5-60 Watt), in order to optimise the precursors fragmentation and to tune selectivity and stability of the deposited polymers. In situ diagnostics (Mass Spectrometry and Optical Emission Spectroscopy) have been used in order to control the deposition processes and analyse the fragmentation steps. The films have been characterised with X-Ray Photoemission Spectroscopy (XPS) and Fourier Transformed Infrared Spectroscopy (FTIR). Surface energy of the coatings has been determined by contact angle measurement. The protein adsorption kinetics has been evaluated with the Quartz Crystal Microbalance (QCM-D) with HSA. The results demonstrate a strong link between monomer fragmentation in the plasma and functional groups retention in the films. By increasing the RF power, the COOH concentration in the films (XPS and FTIR) as well as hydrophylicity, hydrogen bondings and acid-base character decrease while the CO concentration in the plasma phase (MS and OES) increases. At the same time, the dispersive and the polar components of the surface free energy increase. These surface properties have a strong influence on the protein attachment kinetics, as determined by QCM measurements.

Biomaterials
Room: C-201 - Session BI+HS+SS-ThM

Biosensors and Biodiagnostics
Moderator: J. Hickman, Clemson University

8:20am **BI+HS+SS-ThM1 Surface Functionalization for Self-Referencing and Multi-Channel Surface Plasmon Resonance (SPR) Biosensors**, ***J. Ladd***, *C. Boozer, Q. Yu, J. Homola, S. Yee, S. Jiang*, University of Washington

Recently, a novel SPR sensor with on-chip referencing has been realized. In this sensor, one half of the gold sensing surface is covered with a high refractive index overlayer of tantalum pentoxide (Ta2O5). When polychromatic beam illuminates the sensing surface, surface plasmon resonance in the areas with and without the overlayer occur at different wavelengths. Therefore, the reflected light exhibits two dips associated with SPRs in those two areas. When functionalized properly, one of the areas can be used as a specific sensing channel for detection of specific bio-interactions and the other can act as a reference channel for compensation for background refractive index fluctuations. In this work we present a new functionalization approach for these mixed architecture chips. The gold side of the chip is functionalized with a mixed self-assembled monolayer of polyethylene oxide (PEO) and biotin terminated thiols whereas the Ta2O5 side is coated with PEO terminated silanes. The PEO terminated thiols and silanes serve as a protein resistant background, while the biotin-terminated thiols are used to bind streptavidin, which in turn immobilizes biotinylated antibodies. Hence, the gold side of the chip is used for the binding and detection of target analytes and the Ta2O5 side functions as a reference channel that monitors bulk refractive index changes and temperature drift. We have applied this functionalization to an SPR based biosensor and have studied two model systems: mouse IgG and human hCG. In addition, we have quantified and compared the protein resistance of the PEO thiols versus the PEO silanes. This information will help us better compensate for non-specific effects and improve robustness of SPR measurements.

8:40am **BI+HS+SS-ThM2 Chemical Sensing Using Ultra-Fast Micro-Boiling**, ***O. Thomas***, *R.E. Cavicchi, M.J. Tarlov*, National Institute of Standards and Technology

We report a novel liquid sensing method that exploits micro-boiling phenomena on the surface of rapidly heated thin film heaters. The heaters are thin films of platinum and gold-plated platinum that are approximately tens of micrometers in width and hundreds in length. The micro-heaters are immersed in solutions where they are rapidly heated to high temperature with short, 5 - 40 microsecond, square voltage pulses. The temperature-time responses of the micro-heaters are obtained by measuring their resistance during the application of the heating pulse. The bubble nucleation event associated with boiling is signaled in the temperature-time transient by an inflection point that results from a change in heat transfer when a vapor film forms on the heater. Because of the extremely high heating rates, superheating is observed where nucleation temperatures approaching 300°C have been measured for aqueous solutions. The bubble nucleation temperature and average heater temperature during the micro-boiling process have been found to be highly dependent on the surface wettability of the heater, as well as the presence of surfactant molecules. We will report on the use of alkanethiol self-assembled monolayers to investigate the effect of surface wettability on micro-boiling. We will demonstrate that temperature-time transients of hydrophobic SAMs are distinct from those of hydrophilic SAMs and that information on SAM stability can be gleaned from transient data. We will also present preliminary results on using the micro-boiling phenomenon to detect surface binding events such as DNA hybridization and biotin-avidin coupling.

9:00am **BI+HS+SS-ThM3 Nanofluidic and Biomimetic Bioanalytical Systems**, ***G.P. Lopez***, University of New Mexico **INVITED**

This talk will present recent progress on the development of hybrid nano-materials containing synthetic and biosynthetic components for use in bioanalytical applications including separation and biosensing. Examples include the development of mesoporous s ilica microbeads that incorporate functional biomolecular components (e.g., transmembrane proteins in lipid bilayer systems) and stimuli-responsive polymers for the formation of "cell mimics" that preserve biological function in a robust, deterministic, n onliving system. Microscopic beads can be used in a variety of bioanalytical system formats including suspension assays in flow cytometry and microfluidic assays and separations in affinity microcolumns. Several aspects of these bioanalytical systems will be explored including optimization of ligand-receptor pairs for direct transduction of biomolecular recognition, microfluidic considerations, and fluorescence detection principles.

9:40am **BI+HS+SS-ThM5 A Gold Nanoparticle Sensor to Interrogate Biomolecular Interactions in Real-time on a Surface**, *N. Nath*, ***A. Chilkoti***, Duke University

We present a label-free optical technique to study biomolecular interactions in real time on a surface that is based on particle surface plasmon resonance (PSPR). We demonstrate that the absorbance spectrum of immobilized gold nanoparticles on glass exhibits a red shift as well as an increase in the absorbance at peak wavelength as a function of binding of biomolecules at the solid-water interface. The results obtained with the absorbance sensor were compared with those obtained using conventional SPR for fibrinogen adsorption onto a COOH-terminated surface and for the binding of streptavidin to a biotin-functionalized surface. We have also examined the sensitivity and dynamic range of the sensor as a function of nanoparticle size, and found a threefold improvement in sensitivity as the size of the nanoparticles is increased from 13 to 50 nm. This sensor is attractive because of its simplicity: gold nanoparticles are easily prepared with high reproducibility, they can be readily immobilized on glass, and their absorbance spectrum can be easily measured using widely available UV-vis spectrophotometers. Furthermore, this technique should be easily amenable to the design of chips in an array format for application in high-throughput immunoassays and proteomics.

10:00am **BI+HS+SS-ThM6 Evaluation of Methodologies for Arraying a Porous Inorganic Bioassay Support**[1], ***C. Cole***, Nova Research, Inc., *D.B. Chrisey, R.J. Colton, H. Kim, B.R. Ringeisen*, Naval Research Laboratory, *C.R. Tamanaha*, Geo-Centers, Inc., *L.J. Whitman*, Naval Research Laboratory

A membrane-based immunosensor has been developed for the detection of eight biological agents with a response time of <15 minutes and a sensitivity ~3 orders of magnitude higher than conventional ELISAs. The Force Discrimination Biosensor[2] (FDB) uses generically functionalized 0.8 µm-diameter beads to label captured target; a magnetic field gradient removes nonspecifically bound beads, thus improving sensitivity by reducing both background and the incident of false positives. Already demonstrated for single analyte detection, methodologies to array the alumina ultrafiltration membrane for multiplexed detection have been evaluated. One of the biggest challenges is to array hydrophobic antibody conjugates onto porous hydrophilic PEG-biotin surfaces without losing pattern integrity due to lateral wicking. Patterning via a PDMS stamp or mask works reasonably well, but is too cumbersome for the patterning of the large number of membranes needed for practical applications. Instead, a pulsed laser transfer technique developed at NRL has been adapted to pattern antibody conjugates[3] onto PEGylated membranes. With an average element dimension of $(100\ \mu m)^2$ and 200 µm spacing between elements, a 10 x 10 array can be written in 3 mm^2. Such arrays can be patterned to give a single diagnostic for a variety of bacterial, viral, or protein agents without requiring the use of an additional membrane for positive/negative controls. Multiplexed assays for bacterial spores and cells, viruses, and protein toxins have been performed with these filters; results will be presented to demonstrate the application of pulsed laser writing to biosensor patterning.

[1] Supported by the Joint Service Technical Panel for Chemical and Biological Defense.

[2] Lee et al., Anal. Biochem. 287, 261 (2000).

[3] Ringeisen et al., Biomaterials 23, 161 (2002).

10:20am **BI+HS+SS-ThM7 DIOS-MS for Reaction Monitoring and Chemical Analysis**, *Z. Shen*, University of California, San Diego, *G. Siuzdak, M.G. Finn*, The Scripps Research Institute, ***J.E. Crowell***, University of California, San Diego

Desorption/Ionization On Silicon Mass Spectrometry (DIOS-MS) is a new mass spectrometry strategy based on pulsed laser desorption/ionization from a porous silicon surface. DIOS-MS is similar to matrix-assisted laser-desorption ionization mass spectrometry (MALDI-MS) in that it utilizes the same instrument; however, in DIOS-MS, porous silicon is used to trap analytes deposited on the surface and laser radiation is used to vaporize and ionize these molecules, without the presence of any matrix material. We have shown that DIOS-MS can be used for a wide range of small molecules as well as biomolecules at the femtomole and attomole level with little or no fragmentation. DIOS-MS offers many unique advantages including good sensitivity, low background ion interference, and high salt tolerance. We will demonstrate the application of DIOS-MS to small molecule quantitative analysis, high throughput screening, chemical reaction monitoring, enzyme-substrate reaction and inhibition characterization, drug

metabolism studies, and protein identification. We will also discuss aspects of the desorption and ionization mechanisms of DIOS.

10:40am **BI+HS+SS-ThM8 ToF-SIMS Analysis of PNA/DNA Hybridization on Thiolated Biosensor Chips**, ***M. Schröder***, Westfälische Wilhelms-Universität Münster; Germany, *J.C. Feldner, S. Sohn, H.F. Arlinghaus*, Westfälische Wilhelms-Universität, Germany

We have investigated a diagnostic method that uses peptide nucleic acid (PNA) biosensor chips to detect hybridization of unlabeled DNA. Using two different approaches, different PNAs were immobilized onto Au-coated spots with an approximate diameter of 100µm. One method was to immobilize thiolated PNA in a single-step reaction to the Au-surface via an Au-S-bond. The other method was to crosslink the N-terminal end of the PNA to a preformed layer of 11-mercaptoundecanoic acid (MUA) in a reaction consisting of two steps forming an amide bond. These layers were hybridized with complementary and non-complementary unlabeled single-stranded DNAs (ssDNA). Since the backbone of DNA, in contrast to PNA, contains phosphorous, it is possible to identify DNA-PNA-hybrids with time-of-flight mass spectrometry (ToF-SIMS) via DNA-specific phosphate-related ions at the masses 63 amu (PO_2^-) and 79 amu (PO_3^-). In addition to these signals, the deprotonated bases M-H$^-$ were detected in both immobilization approaches. In the case of the two-step-immobilization, it was possible to independently control the different steps by measuring characteristic peaks of MUA-fragments. Due to the manifold control-possibilities, especially variation of surface-density of the immobilized PNA and saturation of the remaining active Au-binding-sites with different thioles, it is possible to optimize hybridization conditions and suppression of uncharacteristic bonding of the ssDNA to the Au-surface. From the obtained data it can be concluded that both PNA immobilization approaches are very promising for designing PNA biosensors and that ToF-SIMS is a useful tool for identifying DNA-PNA-hybrids on these biosensor chips with good discrimination.

11:00am **BI+HS+SS-ThM9 Covalent Attachment and Hybridization of DNA Oligomers at Polycrystalline Diamond Thin Films**, *T. Knickerbocker, W. Yang, W. Cai*, University of Wisconsin-Madison, *J.N. Russell, Jr., J. Butler*, Naval Research Laboratory, *D.M. Gruen, J.A. Carlisle*, Argonne National Laboratory, *L.M. Smith, D. Van der Weide*, ***R.J. Hamers***, University of Wisconsin-Madison

Diamond has a number of unique properties, including a very wide range of electrochemical stability and very good electrical and thermal properties. These properties may make diamond a particularly attractive material to use as a substrate for biological sensors. We have explored the covalent bonding of DNA to several different types of diamond thin films, including free-standing polycrystalline films, thin films of microcrystalline diamond on silicon substrates, and ultrananocrystalline diamond thin films. Starting with H-terminated diamond, we prepared a homogeneous amine-terminated surface using a photochemical attachment processes, optimized using core-level photoemission spectroscopy. These amine-terminated diamond surfaces are then used as a starting point for subsequent attachment of DNA oligomers. The efficiency and selectivity of hybridization have been determined using conventional fluorescence measurements after the surface-bound oligomers are hybridized with fluorescently-tagged complementary and non-complementary oligomers. Our studies show that DNA-modified diamond surfaces show good hybridization properties and good selectivity. More importantly, the DNA-modified diamond surfaces show extremely good stability with repeated hybridizations, and retain this selectivity even after being dried and later reconstituted. This talk will discuss the fabrication of DNA-modified diamond surfaces for biosensor applications, and the differences and similarities between the various forms of DNA-modified diamond thin films.

11:20am **BI+HS+SS-ThM10 Direct Electronic Detection of DNA Hybridization at Surfaces**, ***W. Cai***, *J. Peck, D. Van der Weide, RJ. Hamers*, University of Wisconsin-Madison

We have explored the use of electrical measurements to detect DNA hybridization in a label-free manner at surfaces. Our work has emphasized materials that are compatible with microelectronics, including DNA-modified surfaces of silicon, gold, and diamond. While most previous studies have focused on detection via low-frequency measurements, our work has focused on measurements at high frequencies, from ~10 kHz up to 10 GHz. The use of radio- and microwave-frequencies brings with it reduction in 1/f noise, the possibility of constructing electrically resonant devices for enhanced sensitivity, and the ability to perform single-ended measurements based on reflection instead of transmission. At these high frequencies, the electrical properties are controlled by the capacitance of the electrical double-layer, with some possible contributions from the space-charge region of semiconducting substrates. Using electrochemical impedance spectroscopy, we find a small, but reproducible change in capacitance at the interface when DNA oligomers are hybridized with the complements. By comparing the responses generated when the surface-bound oligos are exposed to matched and mismatched sequences in solution, we can separate the changes in dielectric properties arising from hybridization from other possible sources of systematic error. To enable measurements to be performed with high sensitivity on very small areas, we have constructed a novel heterodyne reflectometer that allows us to measure the dielectric properties of very small interfaces in a manner that is essentially zero-background. To do this, we take advantage of the fact that the electric double-layer is intrinsically nonlinear, and that hybrization and other biological binding processes modify the dielectric properties of the double-layer region. This talk will discuss different schemes for direct electronic detection of DNA hybridization, with particular emphasis on the use of RF and microwave methods.

11:40am **BI+HS+SS-ThM11 Engineered Biointerfaces for Protein Biochip Applications**, ***H.B. Lu***, *M. Mariano, S. Schweizer, H.M. Tran, L.A. Ruiz-Taylor, H. Hong, H.H.J. Persson, R.L. Cicero, P. Kernen, P. Wagner*, Zyomyx, Inc.

Protein biochip technology promises breakthroughs in large-scale protein analysis. Measuring and analyzing protein activities in a highly efficient, miniaturized and parallel fashion requires advanced surface chemistries for reproducible protein immobilization and minimized non-specific adsorption. Controlling the solid-liquid interface of a miniaturized biochip becomes a key step for maintaining protein activity and integrating highly sensitive detection techniques. We present several reactive surfaces engineered for protein biochip applications at Zyomyx. Systematic efforts on designing organic layers on different substrates have been carried out to improve packing density, orientation, and functionality of immobilized capture reagents, as well as to minimize non-specific biomolecule adsorption in complex biological samples. The latter is particularly important for improving detection limits and obtaining meaningful results in multiplex protein assays. To reduce non-specific adsorption and optimize chip performance, we incorporated oligo- and poly-ethylene glycol (EG) molecules in our organic layers that are well known to reduce non-specific protein adsorption. Effects of substrate type, surface coverage, and molecular structure of the assembled organic layers on specific and non-specific interaction of biomolecules with the surfaces are presented. Specificity, loading capacity and detection sensitivity of protein immunoassays using high-density protein arrays configured with these surfaces are demonstrated and discussed.

Biomaterials
Room: C-201 - Session BI-ThA

Cell Patterning to Engineer Function
Moderator: G.J. Leggett, University of Sheffield

2:00pm **BI-ThA1 Analysis of Cell Adhesion Strengthening Using Micropatterned Substrates**, ***N.D. Gallant***, *A.J. García*, Georgia Institute of Technology

Cell adhesion to fibronectin (FN) involves integrin receptor binding and subsequent adhesion strengthening, which includes integrin clustering, interactions with cytoskeletal and signaling components to form focal adhesions (FAs), and cell spreading. We applied micropatterning methods to control FA size and position and decouple FA formation from gross changes in cell morphology in order to analyze the contributions of FA assembly to adhesion strength. Microcontact printing was used to pattern alkanethiol self-assembled monolayers into arrays of circular adhesive islands (2, 5, 10 μm dia.) within a non-adhesive background.[1] NIH3T3 fibroblasts adhered to FN-coated islands and remained constrained to the patterns presenting a nearly spherical morphology. Cells assembled robust adhesive structures that localized to the micropatterned islands and contained typical components of FA. Cell adhesion strength to FN-coated micropatterned islands was quantified using a spinning disk device that applies a well-defined range of hydrodynamic forces to adherent cells.[2] Adhesion strength exhibited significant time- and adhesive area-dependent increases. Comparison of experiments for equivalent contact areas showed a 9-fold increase in adhesion strength over time, independently of cell spreading. These results demonstrate that FA assembly, independently of changes in cell morphology, contributes significantly to adhesion strengthening. This work provides an experimental framework for the functional analysis of FA components in adhesive interactions.

[1]N.D. Gallant et al., "Micropatterned surfaces to engineer focal adhesions for analysis of cell adhesion strengthening," Langmuir (in press).

[2]A.J. García et al., "Force required to break $\alpha_5\beta_1$ integrin-fibronectin bonds in intact adherent cells is sensitive to integrin activation state," J. Biol. Chem. Vol. 273, pp. 1098-10993, 1998.

2:20pm **BI-ThA2 Micropatterning of Polymer Surfaces for Controlled Cell Adhesion and Spreading Processes**, ***C. Satriano***, University of Catania, Italy, *S. Carnazza, S. Guglielmino,* University of Messina, Italy, *A. Licciardello, G. Marletta*, University of Catania, Italy

The prompting of cell adhesion and spreading processes onto polymeric surfaces activated by ion beam irradiation is a phenomenon observed for several polymers. In particular, in the case of carbon-based polymers and silicon-based polymers, the enhancement of cytocompatibility of the ion-irradiated surfaces has been mainly related to the formation of amorphous phases of hydrogenated carbon or SiO_2-like clusters, respectively. In this work the physico-chemical properties of two representative polymers of the two classes above mentioned, i.e., poly(ethyleneterephtalate) (PET) and poly(hydroxymethylsiloxane) (PHMS) were modified in a graded and controlled way with a micrometric spatial resolution. Namely, irradiated patterns with stripes of width ranging between 10 and 100 microns were obtained on the two polymer surfaces by using finely focused ion beams, with a total ion dose of $1x10^{15}$ ion/cm^2. The surface chemical structure and composition of the ion-modified surfaces were characterized by TOF-SIMS and Small Spot XPS, the micro-topography and the morphology were measured by AFM, finally, the surface free energies were calculated by wettability measurements. Fibroblast cells were used to test the cell adhesion and viability on the various micropatterned surfaces. Optical Microscopy was employed to characterize the importance of the lateral resolution effect respectively in PET and PHMS. Epifluorescence Microscopy evidenced the occurrence a specific cell morphology and mitotic activity for the different patterned surfaces. Furthermore, preferential cell alignment effects were observed depending on the type of irradiated polymer.

2:40pm **BI-ThA3 Designing In Vitro Patterned Neuronal Networks**, ***B.C. Wheeler***, University of Illinois, Urbana-Champaign **INVITED**

Through the use of microstamped patterns of polylysine against covalently linked backgrounds of polyethylene glycol, we have been able to maintain patterns of neurons for up to a month in culture. We have demonstrated the ability to use patterning technology in combination with planar microelectrode arrays to confine the neurons to narrow (10 um or 40 um) tracks which intersect the electrodes and to record spontaneous electrical activity (action potentials) from them. Work is in progress to determine how sparse a network can be and still maintain functional electrical activity. This work is intended to provide a technological basis for robust, repeatable and designable neural networks from which one could study basic neuroscience or construct a neural biosensor. Supported by NIH grants R21 NS 38617-01 and R55 RR13320-01 and NSF EIA-0130828. This work is done in collaboration with Dr. Gregory J. Brewer, Dept. of Medical Microbiology, Southern Illinois University School of Medicine.

3:20pm **BI-ThA5 Development of a High Throughput Cell Printing Platform**, *M.V. Deshpande, E.A. Roth,* Clemson University, *A. Gutowska,* Pacific Northwest National Laboratory, ***T. Boland***, Clemson University

High throughput cell printing has a potential to be a very valuable technique in the field of tissue engineering and genomics. A single nozzle cell pen and multi nozzle cell printer have been designed and developed to explore this area. New techniques are being developed to apply these tools for precise placement of cells with high throughput capabilities. The printer nozzles can be loaded with a known concentration of cells in solution or a pre-polymerized hydrogel solution. Pressure created by low power piezoelectric signals will push the cell solution onto a substrate in a programmed design. The cell printer was designed to print cell suspensions to media of any thickness. The body of the printer is made from PMMA with off the shelf printing components (logic board, encoder, etc.). The printer is equipped with a newly designed print head connected to sterile stainless steel hypodermic needles (gauge 30). The needles are individually addressable through piezo driven transducers. Finally, the software drivers were custom written to allow for computer-controlled delivery of single drops. In single-pass mode, the new printer is able to print 80pl drops onto substrata of varying thickness up to 1 inch. Preliminary results indicate a success in developing an array of cells. The cells are alive and healthy as determined by the green stain of the live/dead assay. This indicates the potential of printing small sheets of cells. Other techniques will be investigated to extend the use of the printer to print fluid hydrogel solutions into patterns for use as cell culture templates. Current investigation emphasizes characterizing and comparing temperature sensitive hydrogel mediums. Collagen I, a PLGA based biodegradable gel, and PIPAaM are being investigated. We will present this single cell platform technology and discuss the extension of the technology for two and three-dimensional cultivating systems of varying geometries.

3:40pm **BI-ThA6 Spatially Patterned Tissue for Retinal Cell Transplantation**, *C. Lee*, ***S.F. Bent***, *P. Huie, M.S. Blumenkranz, H.A. Fishman*, Stanford University

Patterning of tissue for selective placement of cells is currently being investigated in a novel treatment for age-related macular degeneration (AMD). The transplantation of human retinal pigment or iris pigment epithelial cells (RPE or IPE) on a carrier substrate is a proposed method for rescuing the diseased retina in AMD. We have examined the use of autologous tissue as a carrier substrate for the cells because it offers several advantages over synthetic substrates. Human lens capsule is readily available through ocular surgery and can coexist in the subretinal space without inducing immune rejection. To control the adhesion and morphology of the RPE cells, we have spatially modified the tissue surfaces using microcontact printing techniques. We have micropatterned inhibitory molecules such as poly (vinyl alcohol) (PVA) on lens capsule and have examined RPE cells subsequently cultured on the surface. We show that micropatterning these molecules via microcontact printing and related flow methodologies confines RPE cells to cuboidal structures that closely mimic the natural RPE layer. The cell inhibition by PVA was found to be stable in culture over a period of weeks. The cells have been successfully patterned on human tissue to circular patches as large as 50 microns and as small as 15 microns in diameter, separated by only a few microns. However, we find that the pattern size strongly affects the probability of cell adhesion and subsequent cell spreading. Overall, micropatterning PVA appears to be a promising and reproducible method for confining cells to high density and to a single morphology. We will discuss the potential for these methods to create a precise and organized transplanted cell layer for the treatment of patients suffering from macular disease.

4:00pm **BI-ThA7 Synaptic Connectivity in Geometrically Defined Neuronal Networks**, ***A. Vogt***, MPI for Polymer Research, Germany, *A. Offenhaeusser,* Research Center Juelich, Germany, *W. Knoll*, MPI for Polymer Research, Germany

One of the major problems in the study of neuronal network behaviour lies in the enormous complexity of the vertebrate brain. A promising approach to this problem is the creation of simplified neuronal circuits in vitro as a model system. A simplified circuit can be achieved by growing neurons on micropatterned substates which impose geometrical constraints upon the

forming network, such that the amount of possible cellular contacts is greatly reduced. Additional advantages of such a system are the clear definition of the connections formed as well as a high reproducability of the network shape. We grew rat embryonic cortical neurons on micropatterned substrates made by microcontact printing of ECM proteins onto a hydrophobic background. The pattern applied was a grid pattern with 6 µm wide lines and nodes that were 14 µm in diameter. The cells aligned with the geometry of the structure and formed simple circuits. Cell density was low enough to observe single cell contacts resulting in the formation of functional synapses along the lines of the pattern; this was shown by triple patch-clamp measurements. The synapses we found did not differ significantly from the synapses found on homogeneous control substrates in average synaptic failure and EPSP height. We therefore believe that our system is suitable as a model for neuronal networks and has multiple potential applications in basic biological research as well as in pharmaceutical testing, neurological implants, neuro-electronics and cell-based biosensors.

4:20pm **BI-ThA8 Oral Keratinocyte Attachment to Chemical Surfaces**, ***R.E. Rawsterne***, UMIST, UK, *G.J. Leggett*, University of Sheffield, UK, *S. Kothari*, UMIST, UK

The control of surface chemistry and topography are key factors in the design and development of next generation biomaterials and prostheses. The importance of surface chemistry has been well established for a variety of cell types, and the importance of surface topography is also gaining momentum. Whilst these are now recognised as being influential in initial cell attachment and growth, and both have been studied independently, there has been little work on examining their combined effects. Furthermore, the effect of these parameters on the behaviour of oral keratinocytes has not been studied. In order to ascertain which chemical functionality would best promote oral keratinocyte attachment, self-assembled monolayers (SAMs) of alkanethiolates on gold with varying chain lengths and acid (COOH), alcohol (OH) or methyl (CH_3) terminal groups were used. To introduce chemical cues to these surfaces SAMs were exposed to UV light through a mask resulting in selective oxidation of specific regions. Following photooxidation, samples were placed in a solution of a contrasting thiol, resulting in the displacement of the oxidised SAM in the exposed region with fresh thiols from solution. Samples exhibiting both single functionality and patterned chemistry were incubated with an oral keratinocyte cell line. For samples with a single functional group, the numbers of attached cells were counted at various time points up to 24h. Attachment to all surfaces was also observed using an inverted microscope and images recorded using a digital camera. It was found that hydroxyl terminated SAMs were the preferred surface for attachment of oral keratinocytes, in contrast to results for the attachment of fibroblasts. This was further investigated by observing the attachment of keratinocytes to patterns comprising of OH/CH_3 and OH/COOH terminated SAMs.

4:40pm **BI-ThA9 The Adaptation of Hydrogel Scaffolds to Three Dimensional Tissue Construction of Cylindrical Vessels**, ***E.A. Roth***, Clemson University, *A. Gutowska*, Pacific Northwest National Laboratory, *T. Boland*, Clemson University

This study investigates the ability of hydrogels to establish patterns for cell growth and their application to the construction of three-dimensional tissues. A variety of hydrogels are being investigated for this application including a collagen based hydrogel and Poly-N-Isopropyl Acrylamide (polyNIPAAm) based copolymers, which undergo liquid-gel transformations in response to temperature changes. The end goal is to construct viable cylindrical vessels that maintain stability, after hydrogel absorption or removal has occurred. A high-throughput cell printing system is under development that allows for accurate cell placement in pre-designed patterns. In this system, bioabsorbable hydrogels and cellular solutions are precisely deposited by needles connected to piezo electric pumps programmed through a software interface. A second method employs a mold consisting of two concentric cylinders, which has been designed to create vessels consisting of smooth muscle cells propagated in a hydrogel matrix. The outer surface of the annulus acts as a structural component during hydrogel stabilization and cellular proliferation. To allow for mold removal, this surface is grafted with polyNIPAAm so that upon slight cooling, the tissue can detach with all cellular junctions intact due to a decrease in hydrophobicity of the polymer. The inner surface of the annulus, composed of an inert nanoporous material, allows for nutrient diffusion from a media reservoir contained in the center of the mold. After sufficient culture time the mold is removed leaving a freestanding cylindrical vessel. Results from both of these methods will be discussed.

5:00pm **BI-ThA10 Integration of Cells and Silicon Devices via Surface Microengineering**, ***J. Hickman***, *M. Das*, *P. Molnar*, Clemson University

The long-term research goal of our group is to learn how to handle and prepare biological cells as components for microdevices and engineered tissues, and then to demonstrate the practicality of this approach by manipulating them to build hybrid systems and engineer functional tissues. The idea is to integrate microsystems fabrication technology and surface modifications with cellular components, with the aim of initiating and maintaining self-assembly and growth into biologically, mechanically and electronically interactive functional multi-component systems. The ability to control the surface composition of an in vitro system, as well as controlling other variables, such as growth media and cell preparation, all play important roles in creating a defined system for hybrid device fabrication. We are using self-assembled monolayers (SAMs) to control the intrinsic and geometric properties of surfaces in contact with these cellular systems. We have used the geometric control of the surface composition afforded us by SAMs to create in vitro circuits of rat hippocampal neurons. We have also demonstrated functional control of these systems by recording the electrophysiological signals on the patterned SAMs in response to stimuli and demonstrated geometric control of synaptic development. We have used geometric only cues to define axonal/dendrite polarity in developing hippocampal neurons which is a key step in creating engineered neuronal networks. Summed together these all represent a growing set of tools for building hybrid cellular systems. We are using this ability to integrate biological systems with silicon-based systems to create cell-based sensors for high throughput drug discovery and functional genomic assays as well as for hybrid neuronal/silicon systems to study biological computation. We are also using what we learn for a more fundamental understanding of cellular development and neuronal regeneration.

Friday Morning, November 8, 2002

Applied Surface Science
Room: C-106 - Session AS+MM+BI-FrM

BioMEMS and Medical Devices
Moderator: K. Healy, University of California, Berkeley

8:20am **AS+MM+BI-FrM1 Characterization of Implant Surfaces**, ***M. Grunze***, University of Heidelberg, Germany **INVITED**

In this talk I will describe my personal recollection of the development of polymer coating (Polyzene FÂ®) for cardiovascular stents from concept to market. The idea was to develop a "stealth" surface coating for metallic stents which reduces inflammation, thrombosis and restenosis of the blood vessels. My talk discusses the design strategies of the polymer, development of the coating process and the necessary Surface Science characterization, protein, cell and bacteria adhesion experiments, the technical certification process, in vivo experiments in animal models, and the problems and successes in starting a new company to market the product. At this time the story is open-ended, since the results of ongoing long term clinical studies were not available at the time this abstract was written.

9:00am **AS+MM+BI-FrM3 Probing the Orientation of Surface-Immobilized IgG by ToF-SIMS**, ***H. Wang***, *D.G. Castner, B.D. Ratner, S. Jiang*, University of Washington

The orientation of a surface-immobilized IgG is crucial for its ability to detect antigen in biosensors. To probe the orientation of a surface-immobilized IgG, two factors are important. One is a powerful surface analysis technique while the other is a well-controlled surface for specific protein orientation. Static time-of-flight secondary ion mass spectrometry (ToF-SIMS) is well suited for this purpose since the sampling depth of ToF-SIMS (1-1.5 nm) is less than the typical dimension of most proteins (4-10 nm). At the same time, IgG orientation can be controlled by appropriately adjusting microenvironments (e.g., surface charges and solution properties). In this work, we apply ToF-SIMS combined with principle components analysis (PCA) to study the orientation of anti-hCG (human chorionic gonadotropin) on two controlled surfaces using its Fab and Fc fragments as references. The controlled surfaces are achieved using self-assembled monolayers (SAMs) with different terminal groups. Results show that the combined ToF-SIMS and PCA technique is able to probe the difference in orientations for ani-hCG adsorbed on different surfaces. In addition, ToF-SIMS results are compared with those from the protein structure. Consistency of these results indicates the reliability of this method.

9:20am **AS+MM+BI-FrM4 TOF-SIMS Analysis to Monitor Coating Processes in Organic and Biological Surfaces**, ***R. Chatterjee***, *B. Lakshmi, M.J. Pellerite*, 3M

Time of Flight Secondary Ion Mass Spectrometry (TOF-SIMS) has proved to be very useful in molecular surface characterization of organic coatings, polymeric systems and biological surfaces. This paper will focus on the application of TOF-SIMS in identifying reaction processes involved in formation of bio-reactive surfaces and organic coatings. In SIMS, absolute quantitative analysis becomes difficult because the ion yield is highly dependent on the morphology and the physical and chemical nature of the surface. Different examples will be used to illustrate how with the use of suitable control experiments, relative quantitative analysis can provide direction in the development of surface modification and surface coating processes. Relative quantitation of TOF-SIMS data was applied to monitor the reaction of aminoacids to different bioreactive surfaces. TOF-SIMS was used to identify presence of different proteins in a multistep sandwich assay. In thin organic coatings, the degree of cure of the silane end group was correlated to the coating durability. Relative quantitation was applied to determine the degree of cure, specify process conditions needed for suitable curing, identify a suitable catalyst to reduce curing times and determine whether lack of cure is the cause of failure. The rate of cure of mono-, bis- and trifunctional silanes, and their effect on the coating durability was investigated.

9:40am **AS+MM+BI-FrM5 Characterization of Protein Interactions with MEMS Devices under Non-Static Conditions**, ***K. Lenghaus***, *J. Dale, D. Henry, J. Hickman*, Clemson University, *J. Jenkins, S. Sundaram*, CFD Research Corporation

The emerging field of micro electromechanical systems (MEMS), when directed to biological applications (environmental monitoring, biosensors etc.), requires an understanding of protein/surface interactions under conditions of flow at low concentrations. Previous protein studies have focussed on adsorption under static conditions and at high concentrations, which can not necessarily be extrapolated to those conditions found in Bio-MEMS under non-static or flow conditions. In an analogous system, the adsorption of proteins to surfaces in in vivo biological systems differs from other adsorption phenomena in that its consequences can be aggressively non-linear, with a biological system's response to minute deviations and changes greatly out of proportion to the magnitude of the change. Thus a relatively small fraction of aggressive sites can induce a response quite out of proportion to their numbers. To study both phenomena we have developed assays to allow enzymes to be quantified at ng/mL levels, and combined with a syringe pump we have created a simple, yet sensitive and robust test bed for protein adsorption under flow conditions. Using this approach, a PEEK capillary was found to have a small number of highly aggressive sites for protein adsorption, corresponding to 5% total surface coverage. These would serve as nucleation sites for further interactions in MEMS devices, and be difficult to detect by other methods. It was further shown that the adsorbed enzymes were in an active state, and this was used to confirm that the rate of desorption from the surface was of the order of 10-4.s-1, corresponding well with values derived from fitting the adsorption isotherm to a computational fluid dynamics model. Thus, studying enzyme adsorption can be used to give several useful insights into the adsorption/desorption behaviour of surfaces at low bulk concentrations of protein as well as generate insights for an in vivo system's protein nucleation behaviour.

10:00am **AS+MM+BI-FrM6 Selective Thermal Patterning of Self-Assembled DNA Monolayers on MEMS-based Microheater Devices**, ***T.H. Huang***, National Institute of Standards and Technology, *N. Ku*, Montgomery Blair High School, *R.E. Cavicchi, M.J. Tarlov*, National Institute of Standards and Technology

We report the selective patterning of self-assembled thiolated DNA probes on gold-coated microheater devices using temperature. The goal of our investigation is to utilize the rapid heating and cooling capabilities of MEMS-based microheaters to prepare biosensing surfaces and to monitor reactions such as DNA hybridization, melting and polymerase chain reaction (PCR). In this study, the self-assembly of thiolated-DNA probes on gold microheater array (four element array) is used as the model system. Modified DNA probes (5' end with disulfide and 3' end with fluorescein) are selectively immobilized onto the gold surface in several steps. First, a passivating layer consists of 1-mercapto-6-hexanol (MCH) is self-assembled onto the gold microheaters. The temperature for one the four heaters is elevated to ca. 200 ^{o}C to drive off the MCH. Then the thiolated DNA probes are deposited onto the freshly exposed bare gold surface. Using this method, one can use temperature to selectively deposit different DNA probes on specific heaters. The presence of the DNA probes on the surface is detected using fluorescence microscopy. In order to use the DNA-microheater surface to monitor DNA melting reactions or PCR (which require cycling to high temperatures), it is important for the probe to be thermally stable at the operating temperatures (i.e. 85 ^{o}C). We will also present results on the thermal stability of thiolated DNA monolayers on gold.

10:20am **AS+MM+BI-FrM7 Soft and Fuzzy Polymer Coatings for Microfabricated Neural Prosthetic Devices**, ***D.C. Martin***, The University of Michigan, *X. Cui*, Unilever, *R. Kim, J. Yang, Y. Xiao*, The University of Michigan **INVITED**

Neural prosthetic devices facilitate the functional stimulation of and recording from the peripheral and central nervous systems. It is important that these implantable devices function in vivo for long periods of time. Bioactive and electrically conductive materials are deposited on the surfaces of neural microelectrode arrays through various means to build a stable interface for better biocompatibility and signal transduction. To mediate the mechanical property differences between the brain tissue and silicon device, integrate the device within tissue and minimize the host reaction, bioactive coatings were developed that can be applied over the whole surface of the silicon micro-devices. One approach that has been developed is electrospinning of protein polymers to form a porous film composed of electrospun nano-scale protein fibers with cell-binding sites exposed. Another ongoing approach has been to coat the device with bioactive hydrogel materials which change volume according to their environment, and therefore integrate the device in the tissue with minimal insertion damage. To stabilize the connection between neurons and the electrode sites and facilitate the signal transduction from electrically conductive metal electrode to the ionically conductive tissue, conductive polymers together with bioactive molecules were co-deposited on the electrode site areas by electrochemical deposition. The coatings presented a fuzzy and conductive

surface which lowered the impedance of the electrode by 1 to 2 orders of magnitude. The bioactive molecules with cell binding ability in the deposited films on the electrode sites were shown to be able to anchor neurons in both in vitro and in vivo experiments.

11:00am **AS+MM+BI-FrM9 Voltage-Dependent Assembly of the Polysaccharide Chitosan onto an Electrode Surface**, *L.-Q. Wu, A.P. Gadre, H. Yi, M.J. Kastantin, G.W. Rubloff, W.E. Bentley, G.F. Payne,* ***R. Ghodssi***, University of Maryland

We examined the assembly of a basic polysaccharide - chitosan - from solution onto electrode surfaces as a result of voltage bias on the electrode. Chitosan is positively charged and water-soluble under mildly acidic conditions, and is uncharged and insoluble under basic conditions. We observed that chitosan is deposited from acidic solution onto the surface of a negative electrode and that the thickness of the deposited layer is dependent upon the deposition time, the applied voltage, and the chitosan concentration. No deposition occurs on the positive or neutral electrode. Once deposited and neutralized, the chitosan layer can be retained on the electrode surface without the need for an applied voltage. Infrared (FTIR) and electrospray mass spectrometry (ES-MS) confirmed that the deposited material was chitosan. The voltage-controlled deposition of chitosan provides a means for anchoring biopolymer material in specific locations in bioMEMS environments, such as encapsulated microfluidic devices fabricated in our laboratory using MEMS-based polymeric materials (EPON SU-8, Polypyrrole and Polydimethylsiloxane). Furthermore, chitosan's amine functionality should enable standard coupling chemistries to be exploited to anchor additional biomolecules (e.g. DNA and proteins) to the surface of bioMEMS devices.

11:20am **AS+MM+BI-FrM10 Alternative Approaches to Microfluidic Systems Design, Construction and Operation**, ***D.J. Beebe***, University of Wisconsin, Madison **INVITED**

Many approaches to the construction of microfluidic systems have appeared in the last few years including glass and silicon etching and bonding, laser machining, micromolding and others. Here we present an alternative approach to the design, construction and operation of microfluidic systems that we call ÂµfluidicÂ tectonics (ÂµFT) that compares to injection molding in cost, but allows for a wide variety of functionality. ÂµFluidic Tectonics utilizes liquid phase photopolymerization, responsive materials and in situ fabrication to achieve elegant yet functional designs. Ultra rapid microchannel fabrication (2 minutes) is demonstrated using off the shelf components (glass microscope slides, polycarbonate top, simple UV lamps and transparency masks). The process eliminates the need for traditional bonding to achieve a closed channel and no master is required (as in elastomeric micromolding). The same basic process has been used to create filtering, flow control, readout (chemical and biological) and mixing components. Thus, the construction platform leads to highly integrated systems by using a single fabrication process and class of materials (photopolymerizable polymers). Closed loop feedback control is demonstrated without the use of electronics. A single structure created in situ from responsive materials performs the sensing and actuation functions. The responsive component senses the local chemical environment and undergoes a volume change in response to changes in the local environment. The volume change is coupled to a valve that regulates the compensating stream providing closed loop regulation. The design flexibility ÂµFT combined with the ease of fabrication and low cost (similar to injection molding) enhances the microfluidic toolbox and broadens the base of potential designers and users by simplifying the construction process and reducing the infrastructure needed to create and use microfluidic systems.

Authors Index

Bold page numbers indicate the presenter

— A —

Abdelhady, H.G.: BI+AS-WeM5, 14
Abramov, M.A.: BI+SS-TuA7, 8
Acharya, A.: BI-WeP16, 17
Ahn, S.J.: BI+SS-TuA4, 8
Al-Bataineh, S.: BI+SS-TuA10, 9
Aliaga-Alcalde, N.: MB+BI+OF-TuA1, 9
Allen, S.: BI+AS-WeM5, 14
Altuzar, V.: BI-WeP21, 18
Andersson, A.-S.: BI-WeP11, 16
Arlinghaus, H.F.: BI+HS+SS-ThM8, 24

— B —

Bachand, G.D.: NS+BI-MoA2, 4
Bagnaninchi, P.O.: BI-TuP2, **11**
Barber, T.A.: BI-WeA10, 20
Barton, D.: PS+BI-WeA1, 21; PS+BI-WeA5, 21
Baruah, T.: MB+BI+OF-TuA3, 9
Bearinger, J.P.: BI-TuP11, 12
Beebe, D.J.: AS+MM+BI-FrM10, **28**
Belghiti, K.: BI-WeP16, 17
Bent, S.F.: BI-ThA6, **25**
Bentley, W.E.: AS+MM+BI-FrM9, 28
Bernstein, N.: MB+BI+OF-TuA3, 9
Bhaduri, S.: MB+BI+OF-TuA1, 9
Blumenkranz, M.S.: BI-ThA6, 25
Boesmans, M.: BI+SS-TuA7, 8
Bohringer, K.: BI-WeP10, 16
Boland, T.: BI-ThA5, **25**; BI-ThA9, 26; BI-WeP6, 15; BI-WeP9, 16
Bonroy, K.: BI+SS-TuA7, 8; BI-TuP18, 13; BI-WeP18, **17**
Boozer, C.: BI+HS+SS-ThM1, 23
Borghs, G.: BI+AS-WeM4, 14; BI-WeP18, 17
Boulmedais, F.: BI-WeA3, 19
Boxer, S.G.: BI+SS-TuM10, 7
Brizzolara, R.A.: BI-WeP17, 17
Buck, M.: BI+AS-WeM1, **14**
Bullett, N.A.: PS+BI-WeA6, **22**
Bunker, B.C.: BI-MoM9, 1; NS+BI-MoA2, 4
Butler, J.: BI+HS+SS-ThM9, 24

— C —

Cai, W.: BI+HS+SS-ThM10, **24**; BI+HS+SS-ThM9, 24
Campitelli, A.: BI+AS-WeM4, 14; BI+SS-TuA7, 8; BI-TuP18, 13; BI-WeP18, 17
Cantalini, C.: HS+SS+BI-WeA10, 21
Carlisle, J.A.: BI+HS+SS-ThM9, 24
Carnazza, S.: BI-ThA2, 25
Castner, D.G.: AS+MM+BI-FrM3, 27; BI+SS-TuM9, 6; BI+VT-MoA3, 3; BI+VT-MoA6, **3**; BI+VT-MoA9, 4; BI-TuP6, 11; BI-WeA10, 20; BI-WeP12, 16; BI-WeP14, 17
Catuogno, C.: NS+BI-MoA6, **5**
Cavicchi, R.E.: AS+MM+BI-FrM6, 27; BI+HS+SS-ThM2, 23
Ceccone, G.: PS+BI-WeA9, 22
Chatterjee, R.: AS+MM+BI-FrM4, **27**
Chen, S.: BI-TuP12, 12; BI-WeP2, 15
Cheng, G.: HS+SS+BI-WeA9, 21
Cheng, T.C.: BI-WeA5, 19
Cheng, X.: BI-WeP10, **16**
Chilkoti, A.: BI+HS+SS-ThM5, **23**; BI+SS-TuA4, 8
Choi, K.-H.: BI+AS-WeM4, **14**; BI-TuP18, 13
Chrisey, D.B.: BI+HS+SS-ThM6, 23
Christou, G.: MB+BI+OF-TuA1, **9**
Cicero, R.L.: BI+HS+SS-ThM11, 24; BI-WeP16, 17
Clemmens, J.: NS+BI-MoA2, 4
Cliquet, P.: BI-WeP18, 17
Cole, C.: BI+HS+SS-ThM6, **23**
Colton, R.J.: BI+HS+SS-ThM6, 23
Cox, E.: BI-WeP18, 17
Craighead, H.G.: NS+BI-MoA5, **4**
Cremer, P.S.: BI+SS-TuM7, **6**
Crowell, J.E.: BI+HS+SS-ThM7, **23**
Csucs, G.: BI-TuP5, 11
Cui, X.: AS+MM+BI-FrM7, 27

— D —

Dahint, R.: BI+AS-WeM6, 14; BI+VT-MoA1, **3**
Dahlborg, D.: BI-WeP1, 15
Dale, J.: AS+MM+BI-FrM5, 27; BI-WeP3, 15
Dalsin, J.L.: BI+SS-TuM4, **6**
Das, M.: BI-ThA10, 26; BI-TuP15, 12; BI-WeP6, 15
Datskos, P.G.: HS+SS+BI-WeA8, 20
Davies, M.C.: BI+AS-WeM5, 14
Dawson, R.A.: PS+BI-WeA1, 21
De Palma, R.: BI-TuP18, 13
De Paul, S.M.: BI+VT-MoA8, 3; BI-TuP11, 12; BI-TuP5, 11
de Vries, A.H.B.: MB+BI+OF-TuA5, 10
Decher, G.: BI-WeA1, **19**
Declerck, P.: BI-WeP18, 17
Dehaen, W.: BI+SS-TuA7, 8
Delerue, C.: NS+BI-MoA7, 5
Deshpande, M.V.: BI-ThA5, 25
Dicke, C.: BI+AS-WeM8, 14
Doran, A.: BI-WeP12, 16
Dubois, Mathieu: NS+BI-MoA7, 5
Dufrene, Y.F.: BI-WeA7, **19**

— E —

Eck, W.: BI+AS-WeM8, 14; BI+SS-TuM3, 6
Elofsson, U.: BI-WeA6, 19
Emmison, N.: BI+SS-TuM11, 7
Eriksson, P.: BI-WeP1, 15

— F —

Fackler, P.J.A.: BI-TuP10, 11
Faure, M.-P.: PS+BI-WeA7, 22
Fauroux, C.M.J.: BI+VT-MoA10, 4
Feldner, J.C.: BI+HS+SS-ThM8, 24
Feller, L.: BI-TuP11, **12**
Finn, M.G.: BI+HS+SS-ThM7, 23
Fischer, D.A.: BI-WeP14, 17
Fisher, E.R.: PS+BI-WeA8, 22
Fishman, H.A.: BI-ThA6, 25
Follonier, S.: BI-WeP16, 17
Foquet, M.: NS+BI-MoA5, 4
Fraser, S.: PS+BI-WeA1, 21
Frederix, F.: BI+AS-WeM4, 14; BI+SS-TuA7, **8**; BI-TuP18, 13; BI-WeP18, 17
Friedt, J.-M.: BI+AS-WeM4, 14; BI-TuP18, 13

— G —

Gadre, A.P.: AS+MM+BI-FrM9, 28
Gallant, N.D.: BI-ThA1, **25**
Garcia, A.J.: BI+VT-MoA5, 3
García, A.J.: BI-ThA1, 25
Gardner, P.: BI+VT-MoA10, 4
Gauckler, L.J.: BI+VT-MoA4, 3
Georgiadis, R.M.: BI+SS-TuA8, **8**
Ghodssi, R.: AS+MM+BI-FrM9, **28**
Giachelli, C.M.: BI-TuP6, 11
Glasmästar, K.: BI-WeP11, **16**
Gold, J.: BI-WeP1, 15; BI-WeP11, 16
Golledge, S.L.: BI-WeA10, 20
Grahek, G.W.: BI-MoM4, **1**
Grandidier, B.: NS+BI-MoA7, **5**
Granick, S.: BI-MoM7, **1**
Greene, M.E.: BI-MoM10, **1**
Greve, J.: MB+BI+OF-TuA5, 10
Griesser, H.J.: BI+SS-TuA10, **9**; BI+VT-MoA8, 3
Gruen, D.M.: BI+HS+SS-ThM9, 24
Grunze, M.: AS+MM+BI-FrM1, **27**; BI+AS-WeM6, 14; BI+AS-WeM8, 14; BI+SS-TuM3, 6; BI-TuP1, 11
Guerin, D.C.: PS+BI-WeA4, **21**
Guglielmino, S.: BI-ThA2, 25
Gutowska, A.: BI-ThA5, 25; BI-ThA9, 26

— H —

Haddow, D.B.: PS+BI-WeA1, 21
Haehner, G.: BI+AS-WeM8, **14**; BI+SS-TuM3, 6
Halter, M.W.: BI+SS-TuM9, 6
Hamers, R.J.: BI+HS+SS-ThM10, 24; BI+HS+SS-ThM9, **24**
Hanein, Y.: BI-WeP10, 16
Harbers, G.M.: BI-WeA10, **20**
Hayashi, T.: BI+AS-WeM6, 14; BI-TuP1, **11**
Healy, K.E.: BI-WeA10, 20
Heilmann, A.: BI-WeP15, **17**
Henderson, J.C.: BI-WeP3, 15
Hendrickson, D.N.: MB+BI+OF-TuA1, 9
Henrickson, S.E.: BI+SS-TuA5, 8
Henry, D.: AS+MM+BI-FrM5, 27; BI-WeP3, **15**
Herrwerth, S.: BI+AS-WeM8, 14; BI+SS-TuM3, 6
Hersam, M.C.: BI-MoM10, 1
Hess, H.: NS+BI-MoA2, **4**
Hickman, J.: AS+MM+BI-FrM5, 27; BI-ThA10, **26**; BI-TuP15, 12; BI-WeP3, 15; BI-WeP4, 15; BI-WeP6, 15; BI-WeP9, 16
Higham, M.C.: PS+BI-WeA3, **21**
Hitchcock, A.P.: BI-WeP12, 16; BI-WeP13, **17**
Hoffman, A.S.: BI+SS-TuA3, 8
Hoh, J.H.: NS+BI-MoA3, **4**
Homola, J.: BI+HS+SS-ThM1, 23
Hong, H.: BI+HS+SS-ThM11, 24
Houston, J.E.: BI-MoM9, 1
Howard, J.: NS+BI-MoA2, 4
Hozumi, A.: BI-WeP19, **18**
Hu, C.: BI+SS-TuA3, 8
Huang, J.K.C.: BI-WeP16, 17
Huang, T.H.: AS+MM+BI-FrM6, **27**
Hubbell, J.A.: BI-TuP11, 12
Huber, D.L.: BI-MoM9, 1
Hug, P.: BI+VT-MoA8, 3
Huie, P.: BI-ThA6, 25
Hyun, J.: BI+SS-TuA4, 8

— J —

Jacob, G.: BI-TuP15, 12
Jalgaonkar, U.: BI-WeP3, 15
Janasek, D.: BI-WeP15, 17
Jandt, K.: PS+BI-WeA9, 22
Jarvis, S.P.: BI-TuP13, 12
Jenkins, J.: AS+MM+BI-FrM5, 27
Jiang, S.: AS+MM+BI-FrM3, 27; BI+HS+SS-ThM1, 23; BI-MoM3, **1**; BI-TuP12, 12; BI-WeP2, 15

— K —

Kageshima, M.: BI-TuP13, **12**
Kam, L.: BI+SS-TuM10, **7**
Kameyama, T.: BI-WeP19, 18
Kanger, J.S.: MB+BI+OF-TuA5, **10**
Kasemo, B.: BI-WeP11, 16
Kasianowicz, J.J.: BI+SS-TuA5, **8**
Kastantin, M.J.: AS+MM+BI-FrM9, 28
Keller, B.: BI+VT-MoA8, 3
Kelly, J.: BI-WeP1, **15**
Kernen, P.: BI+HS+SS-ThM11, 24; BI-WeP16, 17
Kilcher, G.: BI-WeP16, 17
Kim, B.-I.: BI-MoM9, **1**
Kim, H.: BI+HS+SS-ThM6, 23
Kim, R.: AS+MM+BI-FrM7, 27
Kimura-Suda, H.: BI+SS-TuA9, 9
Kinsel, G.R.: BI+SS-TuM5, **6**; BI-TuP17, 12

Klemberg-Sapieha, J.E.: PS+BI-WeA7, 22
Knickerbocker, T.: BI+HS+SS-ThM9, 24
Knoll, W.: BI-ThA7, 25
Kolmakov, A.: HS+SS+BI-WeA9, 21
Korlach, J.: NS+BI-MoA5, 4
Kortus, J.: MB+BI+OF-TuA3, 9
Kothari, S.: BI-ThA8, 26
Krenn, B.: MB+BI+OF-TuA5, 10
Kreuzer, H.J.: BI-MoM5, **1**
Ku, N.: AS+MM+BI-FrM6, 27
Kulkarni, S.: BI+SS-TuA3, 8

— L —

Ladd, J.: BI+HS+SS-ThM1, **23**
Lakshmi, B.: AS+MM+BI-FrM4, 27
Latour, R.A.: BI+VT-MoA5, **3**; BI-MoM4, 1; BI-WeP4, 15
Laureyn, W.: BI+AS-WeM4, 14; BI+SS-TuA7, 8; BI-TuP18, **13**; BI-WeP18, 17
Lavalle, Ph.: BI-WeA3, 19; BI-WeA4, 19
Lavrik, N.V.: HS+SS+BI-WeA8, **20**
Lee, C.: BI-ThA6, 25
Lee, W.: BI+SS-TuA4, 8
Lee, Y.: BI-WeA5, 19
Leggett, G.J.: BI+VT-MoA10, 4; BI-ThA8, 26
Lenghaus, K.: AS+MM+BI-FrM5, **27**; BI-WeP3, 15
Lennen, R.M.: BI-WeP17, **17**
Levene, M.: NS+BI-MoA5, 4
Li, L.: BI-TuP12, **12**
Li, M.: BI+SS-TuM5, 6; BI-TuP17, 12
Licciardello, A.: BI-ThA2, 25
Liu, L.: BI-WeP2, **15**
Lochhead, M.J.: BI-WeP7, **16**
Lopez, G.P.: BI+HS+SS-ThM3, **23**; BI-WeP8, 16
Lozzi, L.: HS+SS+BI-WeA10, **21**
Lu, H.B.: BI+HS+SS-ThM11, **24**; BI-WeP16, 17

— M —

MacNeil, S.: PS+BI-WeA1, 21; PS+BI-WeA3, 21
Maes, G.: BI+SS-TuA7, 8; BI-TuP18, 13
Malmstadt, N.: BI+SS-TuA3, 8
Manning, R.J.: BI+VT-MoA10, **4**
Mariano, M.: BI+HS+SS-ThM11, 24
Marletta, G.: BI-ThA2, 25
Martin, D.C.: AS+MM+BI-FrM7, **27**
Martin, S.M.: BI-TuP6, 11
Martin, T.L.: BI-WeP16, 17
Martinu, L.: PS+BI-WeA7, 22
Matzke, C.M.: NS+BI-MoA2, 4
May, C.J.: BI+VT-MoA9, 4
McArthur, S.L.: BI+SS-TuM9, **6**; BI+VT-MoA9, 4
Meier, L.P.: BI+VT-MoA4, 3
Melendez-Lira, M.: BI-WeP21, **18**
Mendoza-Barrera, C.: BI-WeP21, 18
Messersmith, P.B.: BI+SS-TuM4, 6
Metzger, S.: BI-WeP7, 16
Misakian, M.: BI+SS-TuA5, 8
Mitchell, S.A.: BI+SS-TuM11, **7**
Miyake, J.: BI-TuP13, 12
Miyama, M.: BI-WeP5, 15
Molnar, P.: BI-ThA10, 26; BI-TuP15, 12
Morin, C.: BI-WeP12, **16**; BI-WeP13, 17
Moskovits, M.: HS+SS+BI-WeA9, **21**
Muir, B.W.: BI+SS-TuA10, 9
Mukhopadhyay, R.: NS+BI-MoA3, 4
Muralidharan, G.: HS+SS+BI-WeA8, 20

— N —

Nakamura, C.: BI-TuP13, 12
Napoli, A.: BI-TuP11, 12
Nath, N.: BI+HS+SS-ThM5, 23
Nelson, W.J.: BI+SS-TuM10, 7
Neumann, M.J.: BI-TuP10, **11**
Nishida, J.: BI-WeP5, 15
Nishikawa, K.: BI-WeP5, 15
Nys, J.P.: NS+BI-MoA7, 5

— O —

Offenhaeusser, A.: BI-ThA7, 25

— P —

Pasche, S.: BI+VT-MoA8, **3**
Payne, G.F.: AS+MM+BI-FrM9, 28
Paz, Y.: BI-TuP14, **12**
Pecher, K.: BI+AS-WeM3, 14
Peck, J.: BI+HS+SS-ThM10, 24
Pederson, M.R.: MB+BI+OF-TuA3, **9**
Pellerite, M.J.: AS+MM+BI-FrM4, 27
Perez, T.D.: BI+SS-TuM10, 7
Persson, H.H.J.: BI+HS+SS-ThM11, 24; BI-WeP16, **17**
Pertsin, A.J.: BI+AS-WeM6, 14; BI-TuP1, 11
Peterson, A.W.: BI+SS-TuA8, 8
Petrovykh, D.Y.: BI+SS-TuA9, **9**
Picart, C.: BI-WeA3, 19; BI-WeA4, 19
Pilling, M.J.: BI+VT-MoA10, 4
Poeta, M.: BI-TuP15, **12**; BI-WeP9, 16
Ptak, A.: BI-TuP13, 12

— Q —

Queeney, K.T.: BI-TuP9, **11**
Quincy, D.A.: BI-WeP16, 17

— R —

Ratner, B.D.: AS+MM+BI-FrM3, 27; BI+SS-TuA1, **8**; BI-TuP6, 11; BI-WeP10, 16
Ratner, M.A.: BI-MoM10, 1
Rawsterne, R.E.: BI-ThA8, **26**
Reviakine, I.: BI-TuP5, 11
Rezwan, K.: BI+VT-MoA4, **3**
Richert, L.: BI-WeA4, 19
Ringeisen, B.R.: BI+HS+SS-ThM6, 23
Roberts, C.J.: BI+AS-WeM5, 14
Robertson, B.: BI+SS-TuA5, 8
Roncali, J.: NS+BI-MoA7, 5
Rossetti, F.: BI-TuP5, **11**
Rossi, F.: PS+BI-WeA9, **22**
Rossini, P.: PS+BI-WeA9, 22
Roth, E.A.: BI-ThA5, 25; BI-ThA9, **26**
Royce, C.: BI-TuP9, 11
Rubloff, G.W.: AS+MM+BI-FrM9, 28
Ruiz-Taylor, L.A.: BI+HS+SS-ThM11, 24; BI-WeP16, 17
Russell, Jr., J.N.: BI+HS+SS-ThM9, 24
Ruzic, D.N.: BI-TuP10, 11

— S —

Saavedra, S.: BI+SS-TuM6, **6**
Sailor, M.J.: HS+SS+BI-WeA1, **20**
Samara, M.A.: BI-MoM9, 1
Samuel, N.T.: BI-WeP14, **17**
Santucci, S.: HS+SS+BI-WeA10, 21
Sato, K.: BI-WeP5, 15
Satriano, C.: BI-ThA2, **25**
Schaaf, P.: BI-WeA3, **19**; BI-WeA4, 19
Scholl, A.: BI-WeP12, 16
Schreiber, F.: BI+AS-WeM6, 14
Schröder, M.: BI+HS+SS-ThM8, **24**
Schwartz, J.L.: BI-TuP6, **11**
Schweizer, S.: BI+HS+SS-ThM11, 24
Schwendel, D.: BI+AS-WeM6, **14**
Shamamian, V.A.: PS+BI-WeA4, 21
Shard, A.G.: BI+SS-TuM11, 7; PS+BI-WeA5, 21
Shen, Z.: BI+HS+SS-ThM7, 23
Shimomura, M.: BI-WeP5, 15
Short, R.D.: PS+BI-WeA1, **21**; PS+BI-WeA3, 21; PS+BI-WeA5, 21
Singh, A.: BI-WeA5, **19**
Siuzdak, G.: BI+HS+SS-ThM7, 23
Smith, L.M.: BI+HS+SS-ThM9, 24
Sohn, S.: BI+HS+SS-ThM8, 24
Soler, M.: MB+BI+OF-TuA1, 9
Spatz, J.P.: NS+BI-MoA8, **5**; NS+BI-MoA9, **5**
Spencer, N.D.: BI+VT-MoA8, 3; BI-TuP5, 11
Spohn, U.: BI-WeP15, 17
Stanish, I.: BI-WeA5, 19
Stayton, P.S.: BI+SS-TuA3, **8**
Steitz, R.: BI+AS-WeM6, 14
Stievenard, D.: NS+BI-MoA7, 5
Stuart, S.J.: BI+VT-MoA5, 3; BI-MoM4, 1
Sundaram, S.: AS+MM+BI-FrM5, 27
Sutherland, D.: BI-WeP1, 15; BI-WeP11, 16
Svedhem, S.: BI-WeP1, 15
Szucs, A.: NS+BI-MoA8, 5

— T —

Tabrizian, M.: BI-TuP2, 11; NS+BI-MoA6, 5
Takebayashi, M.: BI-WeP5, 15
Takeda, S.: BI-TuP13, 12
Tamanaha, C.R.: BI+HS+SS-ThM6, 23
Tanaka, M.: BI-WeP5, **15**
Tarlov, M.J.: AS+MM+BI-FrM6, 27; BI+HS+SS-ThM2, 23; BI+SS-TuA9, 9
Tendler, S.J.B.: BI+AS-WeM5, **14**
Teuscher, N.: BI-WeP15, 17
Textor, M.: BI+SS-TuM1, **6**; BI+VT-MoA4, 3; BI+VT-MoA8, 3; BI-TuP11, 12; BI-TuP5, 11
Thissen, H.: BI+SS-TuA10, 9
Thomas, O.: BI+HS+SS-ThM2, **23**
Thundat, T.: HS+SS+BI-WeA8, 20
Thurell, T.J.: BI-WeA6, **19**
Timmons, R.B.: BI+SS-TuM5, 6; BI-TuP17, 12
Tirelli, N.: BI-TuP11, 12
Tokumoto, H.: BI-TuP13, 12
Tomas, S.A.: BI-WeP21, 18
Tonner, B.P.: BI+AS-WeM3, **14**
Tran, H.M.: BI+HS+SS-ThM11, 24
Truica-Marasescu, F.E.: PS+BI-WeA6, 22
Turner, S.W.: NS+BI-MoA5, 4

— U —

Ulman, A.: MB+BI+OF-TuA7, **10**
Urquhart, S.G.: BI-WeP13, 17

— V —

Van der Weide, D.: BI+HS+SS-ThM10, 24; BI+HS+SS-ThM9, 24
van Driel, R.: MB+BI+OF-TuA5, 10
Vautier, D.: BI-WeA4, 19
Voegel, J.C.: BI-WeA3, 19; BI-WeA4, **19**
Vogel, V.: BI+SS-TuM9, 6; NS+BI-MoA2, 4
Vogt, A.: BI-ThA7, **25**
Vörös, J.: BI+VT-MoA8, 3; BI-TuP5, 11

— W —

Wagner, M.S.: BI+VT-MoA3, **3**
Wagner, P.: BI+HS+SS-ThM11, 24; BI-WeP16, 17
Walt, D.R.: HS+SS+BI-WeA5, **20**
Wang, F.: BI-WeP4, **15**
Wang, H.: AS+MM+BI-FrM3, **27**
Wang, J.: BI+SS-TuA8, 8
Wang, W.: BI-WeP9, **16**
Wang, Y.: BI-WeP10, 16
Wavhal, D.S.: PS+BI-WeA8, **22**
Webb, W.W.: NS+BI-MoA5, 4
Weetall, H.H.: BI+SS-TuA5, 8
Wernsdorfer, W.: MB+BI+OF-TuA1, 9
Wertheimer, M.R.: PS+BI-WeA6, 22
Wheeler, B.C.: BI-ThA3, **25**
Whitman, L.J.: BI+HS+SS-ThM6, 23; BI+SS-TuA9, 9; HS+SS+BI-WeA3, **20**
Whittle, J.D.: PS+BI-WeA5, **21**
Wickes, B.: BI-WeP12, 16
Widom, J.: BI-MoM10, 1
Willcox, M.: BI+SS-TuA10, 9
Williams, P.M.: BI+AS-WeM5, 14
Wilson, K.A.: BI+VT-MoA5, 3
Wolf, L.K.: BI+SS-TuA8, 8
Wu, L.-Q.: AS+MM+BI-FrM9, 28
Wu, Y.: BI-WeP8, **16**

— X —

Xia, N.: BI+VT-MoA9, **4**
Xiao, Y.: AS+MM+BI-FrM7, 27

Xu, T.: BI-WeP6, **15**

— Y —

Yang, J.: AS+MM+BI-FrM7, 27
Yang, W.: BI+HS+SS-ThM9, 24
Yee, S.: BI+HS+SS-ThM1, 23
Yi, H.: AS+MM+BI-FrM9, 28
Yokogawa, Y.: BI-WeP19, 18
Yu, Q.: BI+HS+SS-ThM1, 23

— Z —

Zabeida, O.: PS+BI-WeA7, **22**
Zauscher, S.: BI+SS-TuA4, **8**
Zhang, J.: BI+SS-TuM5, 6; BI-TuP17, **12**
Zhang, Y.: HS+SS+BI-WeA9, 21
Zheng, J.: BI-MoM3, 1
Zhou, J.: BI-MoM3, 1
Zipfel, W.: NS+BI-MoA5, 4
Zwahlen, M.: BI+SS-TuM3, **6**