Marine Biofouling Topical Conference

Room: 609 - Session MB+BI-MoM

Biological Interactions at the Marine Interface

Moderator: M. Grunze, University of Heidelberg, Germany

8:00am **MB+BI-MoM1 Surfaces and Signals: Dissecting Surface Properties for Controlling the Settlement and Adhesion of Algae**, ***J.A. Callow**, M.E. Callow*, University of Birmingham, UK **INVITED**

All man-made structures in marine or freshwater environments suffer from the problem of 'biofouling'- the unwanted growth of bacteria (as biofilms), algae (diatoms and seaweeds) and invertebrates (e.g. barnacles, tube-worms). Most current, commercial antifouling strategies use biocide-containing coatings, which are subject to increasingly stringent environmental restrictions. Alternative coating technologies that do not require biocides either invoke 'deterrence' to prevent organisms from sticking in the first place, or the 'non-stick' or 'foul-release' principle to facilitate the detachment of adhered organisms under moderate shear stress. The search for 'environmentally-friendly' solutions has stimulated basic research efforts in an attempt to understand which interfacial properties (e.g. roughness, wettability, charge, friction, elasticity) are important in influencing the adhesion of fouling organisms. This has been facilitated by the advent of novel technologies, such as various forms of lithography, and self-assembly, which enable the production of test surfaces with systematic variations in structure and properties at the micro- and nano-scales. Motile marine organisms such as spores of Ulva, are highly selective in their choice of a suitable surface for settlement. Therefore, one approach to developing practical coatings based upon 'deterrence' is to try to destabilize the surface cue-sensing mechanisms of the organism. The aim of this presentation is to illustrate how spores of the green alga Ulva, and the diatom, Navicula respond to surfaces presenting a range of well-characterised interfacial properties. Examples will be taken from collaborations developed within the ONR Marine Coatings programme and the EC Framework 6 Integrated Project 'AMBIO' (Advanced Nanostructured Surfaces for the Control of Biofouling).

8:40am **MB+BI-MoM3 Cationic Peptide SAMs for Biofouling Studies**, ***B. Liedberg**, P. Nygren, M. Östblom, Y. Zhou, T. Ederth*, Linköping University, Sweden **INVITED**

Positively charged peptides have been synthesized in our laboratory with the purpose of investigating their anti-biofouling potential. The peptides contain a common leading sequence of Cys(Gly)$_2$ where the cysteine residue offers an convenient handle for oriented attachment to gold. The functional part of the peptides (7-mers) consists of alternating ArgTyr, LysTyr, LysTyrGly as well as mixed layers of ArgTyr and a filling Cys(Gly)$_3$ peptide. The peptide layers have been prepared from aqueous solutions and were characterized with respect to layer thickness, conformation and long-term stability in artificial seawater (ASW) using null ellipsometry and infrared spectroscopy. The peptide layers were also examined with respect to settlement of Ulva linza zoospores. The functional ArgTyr, LysTyr, LysTyrGly peptides displayed enhanced settlement as compared to reference surfaces/coatings. Moreover, the settlement on the ArgTyr layer was greatly increased as compared to the other peptide layers, and the amount of settled spores increased with increasing fraction of the ArgTyr. Most importanty, however, the Ulva spores seemed to settle in a side-on conformation on the ArgTyr layer with their flagella (swimming arms) intact. This mode of settlement has not been observed before. Pre-incubation of the Ulva spores in a solution containing the ArgTyr peptide also influenced the subsequent attachment of the spores to polystyrene in a concentration dependent manner. The ArgTyr peptides in solution seemed to stimulate the settlement up to a concentration of ca. 20 µM above which the number of settled spores started to fall. Microscopic examination of the remaining spore solution after settlement suggests that this is due to an increase in spore death. Thus, the Arg residues in the ArgTyr peptide are believed to convey a membrane-associating effect which seems to have a deterring effect upon settling organisms. Complementary experiments on diatoms (a cell surrounded by a silica shell) revealed that these species settle in a non-selective manner on the different peptides. In addition, ongoing experiments using QCM to study vesicle adsorption onto the peptide layers give support to the suggested peptide-membrane association meachanism that resulted in an abnormal, side-on, attachment of Ulva spores. Interestingly, recent preliminary experiments using barnacle cyprids revealed marginal settlement on the ArgTyr peptide layer.

9:20am **MB+BI-MoM5 Mechanics of Barnacle Glue Surfaces and Relation to Foul Release**, ***G. Walker***, University of Toronto, Canada **INVITED**

Recent results concerning the mechanics of barnacle release will be discussed. The fracture mechanics of a whole barnacle will be examined in the context of nanoscale defect formation that is predicted by theory and structures that are observed in the natural cements.

10:20am **MB+BI-MoM8 Understanding Biofouling Mechanisms In Situ: Molecular Level Studies on Polymer Surface Structures in Water and Polymer-Protein Interactions**, ***Z. Chen***, University of Michigan **INVITED**

Biofouling, the growth of barnacles, seaweeds, tubeworms and other marine organisms on the hulls of ocean-going vessels, causes many problems for the US government including extra financial burdens, excessive consumption of energy, and contamination of the environment. Minimally adhesive polymers or polymers from which foulants can be easily removed are being developed as coatings for use in the marine environment. The possibility to use materials with biocides for marine anti-biofouling purposes have also been explored recently. In our group, a second-order nonlinear vibrational spectroscopic technique, sum frequency generation (SFG) vibrational spectroscopy, has been applied to study surface structures of polymer materials which may be developed as fouling control/release coatings in water, to study interactions between polymer surfaces and biological molecules in situ, and to study how biocides which are being developed for marine anti-biofouling purposes interact with cell membranes. Other techniques such as attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), quartz crystal microbalance (QCM), atomic force microscope (AFM), and contact angle goniometer have been used as supplemental tools in such studies. The polymers which have been studied include model polymers such as polymethacrylates, model poly (dimethyl siloxane) (PDMS), commercial PDMS samples, as well as newly developed anti-biofouling polymer coatings. We elucidated that different polymer surfaces exhibit varied restructuring behaviors in water. To understand polymer surfaces in aqueous environments, it is necessary to investigate them in situ. By examining interfacial protein structures, we showed that different polymer surfaces mediate differed molecular interactions with adhesive proteins of marine organisms. Detailed structural information of proteins at the polymer/protein solution interface can be deduced. In addition, we elucidated the molecular interactions between biocides or polymers containing biocides and cell membranes. Such studies provide molecular level information regarding surface - biological molecule interactions, aiding in the design of coatings with improved anti-biofouling property.

11:00am **MB+BI-MoM10 Surface Tension and Bacterial Attachment Revisited: The Effect of Components of Interfacial Tension**, ***L.K. Ista**, B.P. Andrzejewski, K. Artyushkova, D.N. Petsev, G.P. Lopez*, The University of New Mexico

Bacterial adhesion to surfaces is controlled by the relative strengths of interfacial tensions: those between the bacterium and the surface, the bacterium and the liquid and the surface and the liquid. Models of precisely which properties of the substratum and bacteria most profoundly and predictably affect the attachment of bacteria to a sold surface, as well as methods for accurately and predictively measuring these interfacial tensions, abound, but, to date, none is, in and of itself, sufficient to accurately predict bacterial attachement. A well defined and characterized series of self-assembled monolayers (SAMs) of ω-terminated alkanethiolates on gold were used to systematically explore the effect of the various surface energetic components (e.g. polar, non-polar, electron donating and accepting) of solid substrata on the attachment of the marine bacterium, Cobetia marina. The surface energy of the SAM surfaces was calculated from contact angle data obtained from several different solvents and using different models for calculation, to determine which of these methods most accurately predicted bacterial adhesion. It was discovered that a combination of factors, specifically, the hydrophobicity and Lewis acidity of a surface, promoted the greatest adhesion to these surfaces. A model for adhesion of C. marina has been derived using this measurement. The general applicability of this model to organisms of different surface compositions and environmental niches has been explored. General and specific trends for bacterial adhesion will be discussed.

11:20am **MB+BI-MoM11 Interface Chemistry and Mechanics of Barnacles**, *G.H. Dickinson,* Duke University Marine Laboratory*, D. Ramsay, J.N. Russell, Jr.,* U.S. Naval Research Laboratory*, D. Rittschof,* Duke University Marine Laboratory, ***K.J. Wahl***, U.S. Naval Research Laboratory

Barnacles adhere to all kinds of surfaces in the ocean. These animals settle as larval cyprids, and attach by exuding a protenaceous adhesive. Only recently, with the study of soft and transparent release coatings, has the investigation of the adhesive mechanisms of the barnacle base plate become accessible. We are employing a combination of optical and mechanical spectroscopy to understand the near surface properties of barnacles. Here we report on studies of Amphibalanus amphitrite (little striped barnacle) using a combination of micro-Raman and Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopies, Atomic Force Microscopy (AFM), and micromechanical compression testing to investigate base plate chemistry and mechanics. For barnacles grown on release surfaces, two base plate phenotypes are found - those with a 'hard,' thin adhesive morphology, and those with 'gummy' or compliant adhesive morphology. Micro-Raman, ATR-FTIR, and AFM spectroscopies show significant differences in the protein structure and mineralization of hard and gummy. ATR-FTIR spectroscopy of hard and gummy barnacles, with and without seawater present, showed strong time-dependent responses during reattachment. Mechanical differences between hard and gummy base plates were examined using a custom instrumented flat-punch mechanical tester. The base plate mechanical properties of composite moduli are of order 2 to 5 GPa, with statistically lower modulus for gummy barnacles. The overall flexibility of the barnacle base plate was similar for both hard and gummy barnacles, due to compensating morphologies. Release properties will be discussed in relation to base plate morphology and flexibility.

Marine Biofouling Topical Conference

Room: 609 - Session MB+BI-MoA

Control of Marine Bioadhesion

Moderator: G.P. Lopez, University of New Mexico

2:00pm **MB+BI-MoA1 Engineered Polymer Coatings for Foul-Release Applications**, ***J. Genzer***, *A.E. Özçam, K. Efimenko*, North Carolina State University **INVITED**

Marine biofouling is a serious and complex problem resulting in losses of operating efficiency of ships. Current coating technologies derived from copper- and tin-based compounds are being banned because of detrimental effects on marine environment. Hence there is need for developing efficient marine coatings that would possess no ecological concern. In addition to various chemical approaches, surface topography has also been shown to be important for mechanical defense against biofouling. For instance, Hoipkemeirer-Wilson and coworkers reported that topographically corrugated surfaces are capable of reducing biofouling. The degree to which fouling was reduced was found to depend on the dimensions of the geometrical protrusions as well as the chemistry of the surfaces. Because biofouling includes a very diverse range of various species, whose sizes span several orders of magnitude, one single topographical pattern will not likely perform as an effective antifouling surface. Rather, surface corrugations having multiple length scales acting in parallel should be used in designing effective antifouling surfaces. We have developed a method leading to substrate comprising hierarchically wrinkled (H-wrinkled) topographies. These specimens were prepared by first uniaxially stretching poly(dimethylsiloxane) films, exposing them to ultraviolet/ozone (UVO) radiation for extended periods of time (30-60 minutes), and releasing the strain. After the strain was removed from the specimens the surface skin buckled perpendicularly to the direction of the strain. A detailed analysis of the buckled surface uncovered the presence of hierarchical buckling patterns; buckles with smaller wavelengths (and amplitude) rested parallel to and within larger buckles, forming a nested structure. At least 5 distinct buckle generations (G) were detected with their wavelengths ranging from tens of nanometers to a fraction of a millimeter. The method for producing coatings with H-wrinkled topographies may represent a convenient platform for designing foul-release surfaces. Our recent sea water immersion experiments involving testing over an extended time period indicated that these coating are far superior to flat coatings. We will discuss how the H-wrinkle topographies can be applied to make coatings from just about any type of material and offer methodologies for preparing amphiphilic foul-release coatings from commercially available materials. While more work still needs to be done, the initial observations suggest that the H-wrinkled coatings may represent a new and promising platform for fabricating efficient foul-release marine coatings.

2:40pm **MB+BI-MoA3 Nanoscopically-resolved Amphiphilic Surface Features as Non-toxic, Treacherous Terrain to Inhibit Marine Biofouling**, ***K.L. Wooley***, Washington University in Saint Louis **INVITED**

This presentation will detail amphiphilic nanostructured material systems, constructed from a general methodology that involves the kinetic, in situ crosslinking of thermodynamically-driven phase segregated states of polymer assemblies. Macroscopic crosslinked networks composed of amphiphilic nanodomains presented on the surface and dispersed throughout the material are obtained by crosslinking of the assemblies in bulk samples. Of particular interest for these materials are the complex surface topographies and morphologies that allow for the materials to exhibit antifouling characteristics. The crosslinked macroscopic networks have been focused upon compositions that include hyperbranched fluoropolymers and linear poly(ethylene glycol)s, although the compositional profiles are being expanded. The domains or channels that are present throughout the samples offer interesting opportunities for the packaging and release of guest molecules. The nanoscale dimensions and large interfacial surface areas provide for high loading capacities within uniform host environments, but then also promote the release of these guests at significantly reduced temperatures. The uptake and release of guests from hydrophobic vs. hydrophilic, of varying compositions, structures, and sizes, will be discussed. Most recently, unique mechanical properties have also been observed, and these data will also be presented.

3:40pm **MB+BI-MoA6 Basic Surface Properties and Their Influence on the Adhesion of Marine Organisms**, ***A. Rosenhahn***, University of Heidelberg, Germany **INVITED**

The prevention of biofouling is a major challenge for all man made objects which are in long term contact with seawater. In order to systematically develop non toxic coatings, a fundamental understanding of basic surface properties that inhibit or encourage settlement of marine inhabitants is required. Together with biological partner groups within the EU IP AMBIO[1] we investigate the influence of surface properties such as wetting, charge or morphology on the adhesion and on the removal properties for different marine organisms. To tune wetting and chemical surface properties, self assembly is used as highly versatile technique. For preparation of well defined micro- and nanomorphologies, different lithography and multilayer approaches are used. The interaction of different marine inhabitants with these surfaces will be discussed and compared to general protein resistive properties. Although one main focus of this work is inhibition of settlement, also release properties are tested as important measure of adhesion strength. Apart from established ways of evaluating anti fouling properties, we use digital in-line holography as new tool to study and compare the exploration of different surfaces by swimming marine organisms.[2] Following the original idea of D. Gabor,[3] coherent scattering of radiation can be used to record scattering patterns which contain three dimensional information about investigated objects due to the presence of a reference wave. Holography therefore allows the investigation of three dimensional processes e.g. by tracking particles in real time with sub¬micrometer resolution.[4] We use this novel technique to visualize and analyze the motion and exploration behavior of swimming marine organisms towards surfaces with systematically changing properties. The goal of these three dimensional tracking experiments is to gain a more detailed understanding about surface sensing and the early attachment stages of marine organisms.

[1] Ambio : Advanced Nanostructured Surfaces for the control of biofouling, FP6 EU integrated project, http://www.AMBIO.bham.ac.uk/

[2] M. Heydt, A. Rosenhahn, M. Grunze, M. Pettit, M.E. Callow, J.A. Callow, The Jornal of Adhesion, in press

[3] D. Gabor, Nature 1948, 161, 777

[4] W.Xu, M.H. Jericho, H.J. Kreuzer, Opt. Lett. 2003, 28(3), 164

4:20pm **MB+BI-MoA8 Bioresponse to Engineered Topographies**, ***A.B. Brennan***, University of Florida **INVITED**

This study examines hierarchical combinations in polymers that have been used to produce engineered surfaces, which elicit micro-topographical and chemical cues in biological systems. Nature provides complex chemical forms of polymers that are manipulated through both conformational and configurational forms to yield specific functions. Our recent studies have been focused on the design of polymeric surfaces that can be used as models in the study of biological adhesion mechanisms. The recent expansion of bioengineering has increased our need for better models of cellular adhesion and chemical manipulation of surfaces. A process commonly referred to as contact guidance has been shown to modulate cell shape and function in a variety of cell types. Control of endothelial cell (EC) shape using micropatterned chemical substrates is shown in numerous studies by influencing cell adhesion to proteins, which selectively adsorb to the chemical micropatterns. This presentation will focus on the polymer structures that we have been developing for a topographically modified surface with a range of surface energies and bulk modulus values developed through nanostructural modifications on larger microstructures. In this model, we have been able to study the interactions of the biological-induced factors with the polymer chemistry.

5:00pm **MB+BI-MoA10 Development of Environmentally Benign and Durable Nonfouling Marine Coatings**, ***S. Jiang***, University of Washington

Biofouling on ship hulls and other marine surfaces has become a global environmental and economic issue. Traditionally, the best antifouling coating is TBT (tributyltin)-based paint. Due to increased environmental concern, TBT antifouling coatings have been restricted. Non-toxic, fouling-release coatings based on silicone or fluorinated compounds are under development. These coatings are only effective on vessels moving at high speeds. Currently, we are developing environmentally benign, durable, and cost-effective nonfouling coatings, to which marine microorganisms can not attach, as the next-generation marine coatings. In this work, zwitterionic-based materials will be shown to be effective against various marine microorganisms in laboratory and field tests. We have demonstrated for the first time that poly(sulfobetaine methacrylate) [p(SBMA)] and poly(carboxybetaine methacrylate) [p(CBMA)] based materials and coatings are superlow biofouling. In addition, we have explored various

approaches to apply p(SBMA) or p(CBMA) materials onto surfaces and developed several noncoatings for marine applications. Laboratory tests confirmed the excellent performance of sulfobetaine (SB)-based coatings against marine microorganisms (Ulva spores and barnacle cypris larvae). Recently, we developed SB-based paints and spray-coated them onto surfaces covered with an epoxy primer. Initial field tests of these panels clearly demonstrated that our coatings effectively deferred the settlement of hard foulants. Because of their excellent stability and high effectiveness at preventing microorganisms from adhering to surfaces, SB and carboxybetaine-based materials are excellent candidates for marine coatings. The objective of our work is to create products that will effectively defer biofouling under static conditions over a long period of time.

Applied Surface Science

Room: 610 - Session AS+BI+NS-TuM

Surface Analysis and Related Methods for Biological Materials

Moderator: J. Soares, University of Illinois at Urbana-Champaign

8:00am **AS+BI+NS-TuM1 Nano-bio Chemical Image of Single Cells and Tissues for Bio-medical Applications**, ***D.W. Moon***, *T.G. Lee, J.Y. Lee*, Korea Research Institute of Standards and Science **INVITED**

Biochemical imaging of cells and tissues is a basic infra-technology in various bio-medical applications. Instead of conventional labeling methodology for biomolecular imaging with fluorescent dyes, label-free single cell and tissue biochemical imaging methodologies such as a nonlinear optical technique, coherent anti-Stokes Raman scattering (CARS) and an ion beam sputtering analysis technique, Secondary Ion Mass Spectrometry (SIMS) using cluster ion beams were developed. They were used to measure in a complementary manner 2D or 3D biochemical images of various cells and tissues such as Hella cells, adipogenic stem cells, fat liver tissues, cancer tissues, and skin tissues. Preliminary results will be discussed on the following issues. 1) Interactions of fibroblasts with native and denatured collagen thin films were studied with CARS and SIMS. It was extended to study the interactions of fibroblasts with 500 nm nano-fibers and 5 µm micro-fiber made of 40% poly (glycolic acid) (PGA) and 60% collagen. 2) Photoaging effects of skin by UV radiation were studied with SIMS, which showed significant changes in the biochemical imaging of amino acids representing collagen fibers and lipid molecules. 3) It was shown that SIMS imaging of colon cancer tissues has some potential to develop personalized cancer therapy with new drugs. Finally, the present status and future prospects of nano-bio technology based on laser, mass spectrometry, and nanoprobe for biochemical imaging of single cells and tissues at KRISS will be discussed for practical applications in bio, medical, and pharmaceutical researches.

8:40am **AS+BI+NS-TuM3 SIMS Imaging of Polymer Membranes and Single Cells**, ***G. Jiang***, *R. Michel, D.J. Responte, L. Mayorga, K. Greenland, T.N. Davis, T.A. Horbett, D.G. Castner*, University of Washington

The ability to obtain 3-D images of drug distributions in polymers can provide information about drug loading and release profiles. Likewise 3-D images of biological species (lipids, proteins, sugars, etc.) in cells can provide information about the distribution of those species within the cell. With the advent of C_{60} cluster ion beam sources, it is now possible to use time-of-flight secondary ion mass spectrometry (ToF-SIMS) to examine these important biological problems. This study used a dual beam approach (C_{60}^+ for sputtering and Bi_1^+ or Bi_3^+ for analysis) to generate 3-D images from drug (dipyridamole) loaded polyurethane (PEU) films cast onto glass and single cells (yeast and monocytes) adsorbed onto porous polycarbonate (PC) membranes. 3-D images were successfully obtained from all samples. For PEU films without the drug, the intensity of organic fragment ions from the PEU remained constant until the PEU/glass interface was reached, then decreased as the intensity of fragments from the glass increased. In the initial stages of sputter profiling drug loaded PEU films, the intensity of the drug peaks decreased while the intensity of the PEU fragments increased. Then intensities from both components remained relatively constant until the PEU/glass interface was reached. Molecular ions from the drug were readily detected throughout the entire PEU film. ToF-SIMS 2-D and 3-D images of single yeast (size ~ 5 microns) and monocyte (size ~ 10 microns) cells were obtained for cells adsorbed onto the surface of the PC membrane and within the pores of the PC membrane. Fragments from biological species from these cells (e.g., phospholipid at m/z = 184) could be detected in the ToF-SIMS images. These results indicate the possibility of 3-D chemical state mapping of single cells and other biomedical samples with the spatial resolution of a few microns.

9:00am **AS+BI+NS-TuM4 Surface Energy Control Within Copolymer Libraries Synthesised as Micro Arrays for Biological Screening**, *M. Taylor, A.J. Urquhart,* The University of Nottingham, UK, *D.G. Anderson, R. Langer,* Massachusetts Institute of Technology, *M.C. Davies,* ***M.R. Alexander***, The University of Nottingham, UK

There is currently much interest in polymer microarrays in the field of high-throughput materials development.[1,2] Although combinatorial material synthesis is relatively advanced, methods for characterising the surface chemical properties of such libraries are less well developed. We report on methods to characterise the surface chemistry and surface energy of 480 polymers on a microarray formed using on-slide copolymer synthesis. We used X-ray Photoelectron Spectrometry and Time of Flight Secondary Ion Mass Spectrometry to provide surface chemical information from each spot. Water and diiodomethane contact angle measurements were made from individually dosed picolitre volume droplets to estimate surface energy of each copolymer formulation.[3] Such arrays provide extra challenges for characterisation due to the large sample numbers, small sample size and increased data volume. Here, we will focus on the correlations determined between the monomer structures and the surface energy. The information XPS and SIMS can provide on the actual surface chemistry is presented and contrasted to the bulk surface chemistry. We highlight the ability to tune the surface energy using certain polymerised monomer combinations by varying their relative concentrations. This has great utility in controlling the biological response to polymer surfaces.

[1] J. A. Hubbell, Nature Biotechnology 2004, 22, 828.
[2] D. G. Anderson, S. Levenberg, R. Langer, Nature Biotechnology 2004, 22, 863.
[3] Taylor, M.; Urquhart, A. J.; Zelzer, M.; Davies, M. C.; Alexander, M. R., Picolitre water contact angle measurement on polymers. Langmuir Letters (2007, in press).

9:20am **AS+BI+NS-TuM5 Surface Characteristics of Listeria Monocytogenes Mutants with Variable Pathogenicity Levels**, ***N.I. Abu-Lail***, *B.-J. Park*, Washington State University

Despite being an important food-borne pathogen, L. monocytogenes in fact comprises a diversity of strains with varying virulence. Whilst many strains of L. monocytogenes have pathogenic potential and can result in disease and mortality, others have limited capability of establishing infections and relatively avirulent. Although very important, the question of how the composition of the bacterial surface and the properties of bacteria vary between strains that have different level of virulence at the molecular level needs to be answered. To answer this question, interaction forces between five different L. monocytogenes mutants that vary in their virulence and a model surface of silicon nitride were investigated using atomic force microscopy (AFM). Adhesion measurements between the strongest Listeria mutant and silicon nitride reveled that although both surface polysaccharides and surface proteins contributed significantly to the total adhesion, polysaccharides contribution (1.0 ± 0.2 nN) was larger than that of proteins' contribution(0.38 ± 0.1 nN). Adhesion forces were also dependent on the pH value of the solution, temperature, and media type. Experiments on intermediate virulence mutants and avirulent mutants are currently ongoing. Successful completion of these experiments will improve our understanding of the main molecular differences between virulent and avirulent strains of L. monocytogenes. Such findings would be very important, because it will allow for the first time and at a molecular level, to define a criteria that can distinguish virulent L. monocytogenes' strains from avirulent ones and therefore reduce unnecessary recalls of food products and help in preventing disease outbreaks.

9:40am **AS+BI+NS-TuM6 First Observation of Charge reduction and Desorption Kinetics of Multiply Protonated Peptides Soft Landed onto Self-assembled Monolayer Surfaces**, ***O. Hadjar***, *J.H. Futrell, J. Laskin*, Pacific Northwest National Laboratory

Soft-landing (SL) of hyperthermal ions onto semiconductive surfaces is a promising approach for highly-selective preparation of novel substrates using a beam of mass-selected ions. In addition, controlled deposition of complex ions onto surfaces presents a new approach for obtaining molecular level understanding of interactions of large molecules and ions with a variety of substrates relevant for biology and catalysis research. In this work we present a first study of the kinetics of charge reduction and desorption of peptide ions soft-landed onto a fluorinated self-assembled monolayer (FSAM) surface at hyperthermal energy (40 eV). An in situ 8 keV Cs^+ secondary ion mass spectrometry (SIMS) in a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer is used. Doubly protonated peptide ions are produced by electrospray ionization, mass-selected and transferred to the surface. The experiment allows the Cs^+ beam to merge with the peptide ion beam on the surface facilitating real time soft landing process monitoring. The surface is regularly probed using SIMS

during and after ion soft-landing. All peptide-related peaks in SIMS spectra show a gradual increase during the soft-landing. Rapid decay of the $[M+2H]^{2+}$ signal accompanied by increase of the $[M+H]^{+}$ signal is observed after soft-landing is stopped. The $[M+H]^{+}$ signal maximizes 2-3 hours after the end of the soft-landing and shows a relatively slow time decay at longer delay times. Several peptide fragments followed a very different kinetics behavior showing very slow, almost linear decay after soft-landing. We attribute this time signature to fragments that originate from neutral peptide molecules on the surface. Other peptide fragments show a mixed behavior suggesting that they are formed from different charge states of the soft-landed peptide ions. Our results demonstrate for the first time that various peptide-related peaks follow very different kinetics, signatures for doubly protonated, singly protonated and neutral peptides retained on the surface. The experimental results are in excellent agreement with a simple kinetic model that takes into account charge reduction and desorption of different species from the surface. The kinetic modeling allowed us to obtain for the first time desorption and charge exchange rate constants for different peptide species on the surface.

10:40am **AS+BI+NS-TuM9 Ultra Fast Mid Infrared Spectroscopic Imaging for Biomedical Applications**, ***J. Phillips***, *H. Amrania, J. Plumridge, M. Frogley*, Imperial College London, UK

We discuss the potential biomedical applications for a unique infrared spectroscopic micro-imaging system. A table top tuneable solid state laser has been coupled to a commercial infrared microscope to create a unique mid-IR imaging tool. By integrating with a modified high resolution infrared camera that has previously only been available to the military market, we have constructed a broadband imaging system capable of performing diffraction limited spatially resolved spectroscopy of biological specimens. The narrow line-width of the laser allows us to take spectra at a resolution of 20cm-1. A polymer film sample with a micron scale structure has also been imaged in reflective mode to resolve details down to 8 microns in size. We also discuss results from spectrally imaging cancerous cervical tissue samples. The high peak power of the laser (10MW) offers signal to noise levels previously unobtainable with stand-alone laboratory based commercial instruments. This coupled with a short pulse duration will for the first time enable time resolved imaging at a 100psec resolution.

11:00am **AS+BI+NS-TuM10 X-ray Spectromicroscopy and Ion Spectroscopy to Evaluate a Blend of Poly(L)lactic Acid and Fluorine End-capped Poly(L)lactic Acid**, ***D. Wells***, *J.A. Gardella*, University at Buffalo

Blending polymers is a versatile method for tuning the physical and chemical characteristics of a material such as strength, thermal stability, optical properties, and degradation rates. As the field of nanomaterials continues to grow it is essential to be able to evaluate the microstructure of polymeric materials as well as to characterize the chemistry that occurs at the interfaces of blended polymer films. Two techniques capable of such analysis are scanning transmission X-ray microscopy (STXM) and imaging time of flight secondary ion mass spectrometry (ToF-SIMS). STXM is a spectromicroscopy technique, that is, it combines both imaging and chemical spectral information. Recent advancements in cluster primary ion sources for ToF-SIMS have extended the range of its applications. The system of primary interest in this work is a blend of poly(L)lactic acid (PLLA) with fluorine end-capped poly(L)lactic acid (F-PLLA). This material has potential as a drug delivery device whose degradation could be controlled by changing the ratio of hydrophobic F-PLLA to hydrophilic PLLA. It is known that the fluorine containing component will preferentially surface segregate.[1] By reducing the concentration of F-PLLA we predict that we can create lateral surface segregation as well as vertical segregation. Both STXM and ToF-SIMS generate images containing chemical information and are useful to evaluate lateral phase segregation. Our intent is to use these two techniques as the primary means to evaluate the effects of changing the ratio of F-PLLA to that of pure PLLA.

[1]Won-Ki Lee, I. L., Joseph A. Gardella Jr., Synthesis and Surface Properties of Fluorocarbon End-Capped Biodegradable Polyesters. Macromolecules 2001, 34, (9), 3000-3006.

11:20am **AS+BI+NS-TuM11 Influence of Molecular Environment on ToF-SIMS Detection of Bio-Active Molecules on Self-Assembled Monolayers**, ***Z. Zhu***, Pacific Northwest National Laboratory

Bio-active molecules can be immobilized on solid substrates to form a monolayer or sub-monolayer. Because interactions between bio-active molecules are typically special, this structure is very useful in bio-recognition. So far, it has been widely used in bio-analysis or disease diagnosis. Alkanethiol self-assembled monolayer (SAM) on Au substrate is one type of commonly used solid substrate due to its versatile surface properties. During the last decade, time-of-flight secondary ion mass spectrometry (ToF-SIMS) has proven one of the most convenient techniques to detect sub-monolayer of organic molecules on alkanethiol SAMs. We have earlier described the possibility of quantitative detection of peptide molecules on COOH-terminated SAMs. However, we found that molecular environment greatly affect the signal intensity. For example, Au+ signal from -S(CH2)2(CF2)9CF3 film is much stronger than Au+ signal from S(CH2)11CO2H film. Therefore, quantitative comparison of the density of bio-active molecules on different SAMs by ToF-SIMS is difficult unless effect of molecular environment can be quantitatively considered. In this work, a number of bio-active molecules were deposited on -S(CH2)11CH3, -S(CH2)10OH, -S(CH2)10CO2H, and -S(CH2)2(CF2)9CF3 films with similar density, and ToF-SIMS measurements were made. Two major factors are found to affect SIMS signal intensity. Firstly, electron-attraction organic functional groups are found to enhance positive ion signals but depress negative ion signals. For example, positive ion signals are enhanced on -S(CH2)2(CF2)9CF3 film but negative ion signals are depressed. In addition, active H-atoms such as those from COOH groups are able to enhance signal of positive molecular ions since they are normally protonated.

11:40am **AS+BI+NS-TuM12 Advances in Organic Depth Profiling Using Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) under Optimized Ion Beam Conditions**, *H.-G. Cramer, T. Grehl,* ION-TOF GmbH, Germany, *N. Havercroft,* ION-TOF USA Inc., *F. Kollmer, R. Moellers,* ***E. Niehuis***, *D. Rading*, ION-TOF GmbH, Germany

Depth profiling of inorganic materials has been one of the most important applications of SIMS in general, and more recently also of TOF-SIMS. In contrast, depth profiling of organic materials has always suffered from the fact that high-mass molecular information, to a large extent, is rapidly lost under high-dose sputtering conditions. With the advent of cluster ion beams, however, more and more examples of successful organic depth profiling have been presented, such as C_{60} profiling of PMMA, PLA, etc. On the other hand, it also became obvious that the projectiles and conditions commonly used were not successful for profiling of every organic material analyzed. In this paper we used the so-called dual beam mode of depth profiling to start a systematic investigation of organic depth profiling with a TOF-SIMS instrument. Similar to the case of inorganic profiling, we found the dual beam mode beneficial because sample erosion and the sample analysis are decoupled and can be independently optimized. We applied different primary projectiles, such as C_{60}, Bi_n cluster ions, O_2 and Cs with a wide range of impact energies to a variety of organic specimens. The results will be discussed with respect to the specificity of the detected ions, their yields, the damaged and removed sample volume per primary ion, and classical figures of merit such as depth resolution.

12:00pm **AS+BI+NS-TuM13 Fragment Free Mass Spectrometry for Bio-Molecular Surfaces with Size Selected Cluster SIMS**, ***J. Matsuo***, *S. Ninomiya, K. Ichiki, Y. Nakata, T. Aoki, T. Seki*, Kyoto University, Japan

Polyatomic and cluster ions have been utilized for bio-molecular analysis as the primary ion beam for SIMS. Enhancement of sputtering and secondary ion yields, and the capability for depth profiling of bio-materials have been reported for cluster ions, and are due to the effects of multiple collisions and high-density energy deposition of such ions on solid surfaces. In bio-molecular analysis, not only molecular ions, but also fragment ('daughter') ions are usually observed in the mass spectra, and this makes interpretation of the spectrum difficult. Therefore, reducing fragment ions is very important especially for practical applications. These phenomena strongly depend on cluster size, which is a unique parameter, and one of the fundamental questions is what size of cluster ion is most appropriate for bio-SIMS. To date there have been very few studies on the effect of size on secondary ion emission from bio-molecules. We have examined the size dependence of the secondary ion emission from amino acid, sugar and small peptide films with large cluster ion (N>100) by using the double deflection technique. When the total energy of the cluster ion is fixed, the secondary ion emission (SI) yield of molecular ions increases with size due to the non-linear effect. However, when the cluster size is too large, the SI yield is gradually diminished, because the energy per atom becomes too low to emit secondary ions. The maximum molecular SI yield from amino acid film was obtained for Ar clusters with the size of a few hundred at the energy of 20keV. The ratio of fragment ions to molecular ions was also measured as a function of cluster size. The ratio decreases quite rapidly with increasing the cluster size. When the cluster size was larger than 1000, very few fragment ions were observed in the mass spectrum. In this case, each incident Ar atom has kinetic energy of a few eV, which is comparable to the bonding energy of peptides. Ultra-low energy SIMS can be realized by using large cluster ions. The size effect in secondary ion emission and damage cross-section will be discussed.

Biomaterial Interfaces

Room: 609 - Session BI-TuM

Proteins at Interfaces

Moderator: D.G. Castner, University of Washington

8:00am **BI-TuM1 Inter-Domain Structural Flexibility and Biological Activity of Pro-Matrix Metalloproteinase-9 Revealed by Single-Molecule AFM Imaging**, *G. Rosenblum, **S.R. Cohen**, J. Frenkel,* Weizmann Institute of Science, Israel, *N. Slack,* Veeco Metrology Division, Santa Barbara, *I. Sagi*, Weizmann Institute of Science, Israel

The multi-domain enzyme pro-matrix metaloproteinase-9 (pro-MMP-9) is recognized as playing a key role in tumor biology, autoimmune diseases, and vascular pathology. This enzyme cannot be crystallized and hence the only structural information available is of the two isolated terminal domains. Until now, structure of the vital linker domain that connects these terminal domains was unknown. A lack of reliable means to bind the protein to the surface has plagued previous structural characterization by high-resolution AFM imaging. In order to obtain high-quality AFM images of the small protein, novel amine-modified surfaces were employed to immobilize the protein during the extensive rinsing required for removing features due to buffer salts. AFM images presented in this work provide the first definitive confirmation of the multi-domain structure, wherein two terminal domains are connected by a linker segment. Parallel analysis of a mutant lacking the linker showed a less extant shape. Statistical analysis of the AFM images revealed differences in both heights and lengths between the native and mutant proteins, and provided evidence that the linker imparts significant conformational freedom to the molecule, which is likely important in its biological functioning. Biological functioning was further probed, by examining interaction of the enzyme with collagen. Molecular modeling based on the SAXS data provides complementary supporting data.

8:20am **BI-TuM2 Sum Frequency Generation Vibrational Spectroscopic Studies in the C-H, O-H, N-H, and Amide I Regions of Model Peptides at Solid-Liquid Interfaces**, ***R.L. York**, G. Holinga, W.K. Browne, C. Hahn,* Univ. of California, Berkeley and LBNL, *D.R. Guyer,* LaserVision, Inc., *K.R. McCrea, R.S. Ward,* The Polymer Technology Group, *G.A. Somorjai*, Univ. of California, Berkeley and LBNL

We have developed a library of small, model peptides and have examined their interfacial structure at model hydrophobic and hydrophilic surfaces using surface-specific sum frequency generation vibrational spectroscopy (SFG). A fourteen amino acid peptide containing hydrophobic leucine (L) and hydrophilic lysine (K) residues was synthesized and characterized. This amphiphilic α-helical peptide has sequence Ac-LKKLLKLLKKLLKL-NH2 (LK14). SFG spectra in the C-H, O-H, and N-H region reveal that at a hydrophobic deuterated polystyrene surface, methyl groups from the hydrophobic leucine residues are ordered at the interface, while the hydrophilic lysine residues adopt a random orientation (presumably due to lack of interaction with the surface). When adsorbed onto a hydrophilic silica surface, the SFG spectra reveal a completely different molecular orientation: the methyl groups now appear to have a random orientation, and the N-H groups of the lysine side chains and/or peptide backbone are now ordered. A study of the influence of the ionic strength of the solution on the structure of LK14 revealed the following results: the LK14 peptide was α-helical in solution at high ionic strength but random coil at low ionic strength. Furthermore, leucine side chain ordering on hydrophobic surfaces was not perturbed by ionic strength changes, but N-H ordering on hydrophilic surfaces had a strong dependence on ionic strength. More recently, we have developed a new optical parametric amplifier (OPA) utilizing lithium thioindate (LIS) to study the Amide I mode of the peptide backbone. LIS provides high IR energy (~175 µJ at 1500 cm-1, ~375 µJ at 2000 cm-1), a high damage threshold, and good beam quality. The high energy output of LIS allows for the study of interfacial peptide structure without having to use a total internal reflection geometry. Using this new OPA, we have seen evidence for α-helical peptide structure at both hydrophobic and hydrophilic surfaces (at high ionic strengths). Additionally, there appears to be evidence for α-helical structure of the LK14 peptide at hydrophobic surfaces in low ionic strength solutions. We are currently examining lysine homopeptides, collagen-like peptides, new experimental geometries, more biologically relevant surfaces (such as HEMA and polymers with Surface Modifying Endgroups) and molecular dynamics simulations of peptides at interfaces to aid interpretation of experimental data.

8:40am **BI-TuM3 Thin Hydrogel Layers on Biomedical Polymers - Biological Responses and Effects on Protein Adsorption Studied by Mass Spectrometry**, ***D.L. Elbert***, Washington University **INVITED**

Medical devices used in contact with blood often contain features that are on the order of microns, (e.g. the struts on stents). Application of thin polymeric coatings on stents is feasible for the release of antiproliferative agents. However, the use of hydrogel coatings in this application is limited by a number of factors. Thin hydrogel coatings may be difficult to apply on complex geometries. The large volume of water in the hydrogel also limits the amount of drug that can be delivered from the coating. To address the first issue, we previously synthesized a copolymer of polylysine and polyethylene glycol (PLL-g-PEG) that self-assembles on negatively charged surfaces. We demonstrated that very thin yet stable layers of water-soluble polymer reduce biological responses, both in vitro and in vivo. We followed these experiments with investigations into the uses of layer-by-layer strategies, however, practical utility of these films is limited by time-prohibitive methods of fabrication, and the formed films may be too thin for drug delivery. We have addressed these shortcomings in two ways. Rather than delivering drugs directly, we are incorporating an enzyme into hydrogels. The enzyme produces a biologically active molecule (sphingosine 1-phosphate) from a precursor already present in blood (sphingosine). This molecule causes endothelial cell chemotaxis and inhibits smooth muscle cell migration. Additionally, we are producing multilayer films from nanogels that are formed by crosslinking PEG-vinylsulfone with albumin (average particle sizes 40 - 80 nm). Even a single layer of the nanogels covalently-reacted with RFGD-modified PET greatly reduces cell adhesion. Finally, in characterizing protein adsorption on thin hydrogel films, it is important to know not only the amount of adsorbed protein but also the conformations adopted by the adsorbed proteins. To study this, we have developed a proteomics-based strategy to detect differences in the exposure of lysine residues following adsorption. Our studies demonstrated an increased accessibility of lysine residues in fibrinogen adsorbed from low concentration solutions, which correlated well with the increase in the spread area of fibrinogen as measured at the same solution concentrations by OWLS. Overall, tremendous challenges and opportunities exist for producing thin surface coatings that resist non-specific biological adhesion and deliver drugs to control the biological response.

9:20am **BI-TuM5 Development of a Molecular Modeling Program Specifically Designed for the Simulation of Protein Adsorption to Biomaterials Surfaces**, ***R.A. Latour**, P. Biswas,* Clemson University, *B.R. Brooks,* Laboratory of Computational Biology - NIH, *S.J. Stuart*, Clemson University

Although it is well understood that cellular responses to biomaterial surfaces and substrates for tissue engineering and regenerative medicine are primarily governed by the bioactive state of adsorbed proteins, very little is known regarding the molecular-level events involved in these processes. Without this understanding, surface design can only be approached by trial and error. Molecular simulation provides a means to overcome this problem by providing an approach to directly investigate protein-surface interactions at the molecular level. Molecular simulation methods have already been successfully developed and widely applied for the study of protein folding and for drug design. However, these methods are not suitable for protein-surface interactions studies as it involves solid-liquid multiphase interactions which must be addressed specifically. Also, force-fields specifically designed for solid or liquid phase are not easily transferable. We are therefore working to develop capabilities within the CHARMM molecular simulation program to specifically adapt it for the simulation of protein adsorption processes to biomaterials surfaces. In particular, capabilities are being developed to control the solid phase, the solution phase, and the interactions between them with three separate force fields, thus enabling the molecular behavior of each phase of the system to be accurately represented. While force field parameters for proteins in solution and various solid materials have previously been developed and validated, parameters for the interactions between proteins in solution and functional groups presented by a solid surface have not. As an integral part of this program, we are therefore also generating experimental data on peptide-surface interactions for a wide range of amino acid residues and polymer-like functional groups for the design and validation of an interfacial force field for use in the developed program. In this presentation, we will describe the modifications in the CHARMM code and results exhibiting the usefulness of this hybrid force field approach for the simulation of peptide and protein interactions with a solid surface. Once fully developed, this approach holds promise to provide the biomaterials field with an exciting new tool to proactively design biomaterials surfaces to direct cellular response by controlling the bioactive state of adsorbed proteins with broad application in biomedical engineering and bionanotechnology.

9:40am **BI-TuM6 Switching the Force between a Hydrophobic Probe and Self-Assembled Monolayers on Gold by Changing the Ionic Strength**, ***N. Bonnet****, D. O'Hagan, G. Hähner*, University of St Andrews, Scotland, UK

Alkanethiol based self-assembled monolayers (SAMs) have seen an ever increasing interest since they were first introduced more than two decades ago. Part of their attraction is due to the ease of their preparation in combination with the great flexibility they offer to create concentrated planes of functionality by modification of the surfactant molecules. One prominent example are oligo(ethylene glycol) (OEG)-terminated alkanethiol SAMs. They have been shown to resist the non-specific adsorption of some proteins and hence are of significant interest in the life sciences and related areas. Inspired by their protein repelling properties several studies were carried out to determine the underlying mechanisms and the interactions involved. The forces measured with a hydrophobic probe (attraction or repulsion) on these films were found to correlate with the protein adsorption properties (adsorption or resistance to adsorption). The forces indicated the existence of an electrical double layer, suggesting that an electrostatic component is involved. This was confirmed by theoretical calculations. We report on surfactant films based on OEG modified alkanethiol SAMs that can switch between an attractive and a repulsive force with hydrophobic probes depending on the ionic conditions of the environment. The ionic strength is an external parameter that can be easily controlled. The films are interesting for the reversible immobilization of hydrophobic (nano)particles and in colloidal chemistry. Similar surfactants might become important in bio-related fields and in connection with biosensors since they have the potential to adsorb/immobilize proteins reversibly if the switch can be tailored to occur in an ionic range that is compatible with proteins.

10:40am **BI-TuM9 When Good Cholesterol Goes Bad: Proteins at the Water:Lipid Interface**, ***J.W. Heinecke***, University of Washington **INVITED**

Apolipoproteins are amphipathic alpha helical proteins that play a key role in lipid transport in biological systems. HDL - the good form of blood cholesterol that protects against heart disease - is a complex of apolipoprotein A-I (apoA-I), free cholesterol, phospholipids and neutral lipids. Posttranslational oxidative modifications of apoA-I have been proposed to play a pathogenic role in atherosclerosis. Quantifying oxidized amino acids in atherosclerotic tissue proteins by isotope dilution gas chromatography mass spectrometry (GC-MS) has been used to assess oxidative stress in vivo and to investigate the biochemical pathways that contribute to inflammatory disease. These studies have identified myeloperoxidase, a heme protein secreted by activated phagocytes, as one important pathway for oxidizing HDL in the human artery wall. Electrospray ionization tandem mass spectrometry (ESI-MS/MS) coupled with liquid chromatography is rapidly emerging as a powerful technique for pinpointing sites of amino acid oxidation within apolipoproteins. Oxidants generated by myeloperoxidase lead to the site specific oxidation of apoA-I, and these posttranslational modifications are associated with major impacts on the biological function of the proteins. We have recently used 2-dimensional liquid chromatography ESI-MS/MS to test the hypothesis that proteins implicated in inflammation might be enriched in the HDL of subjects with coronary artery disease (CAD). Our observations suggest that HDL carries a unique cargo of proteins in CAD subjects and that certain of these proteins might make previously unsuspected contributions to the anti-inflammatory properties of HDL.

11:20am **BI-TuM11 In situ Quartz Crystal Microbalance Studies of Multi-Layer Glucagon Fibrillation at the Solid-Liquid Interface**, ***M.B. Hovgaard****, M. Dong,* University of Aarhus, Denmark*, D.E. Otzen,* University of Aalborg, Denmark*, F. Besenbacher*, University of Aarhus, Denmark

The ability of certain polypeptides to form amyloid aggregates and their subsequent deposition at plague sites has been associated with multiple protein folding disorders.[1] As in vivo conditions associated with amyloid fibrillation are often dictated by surface and lipid membrane phenomena, the study of amyloid fibrillation at the solid-liquid interface represents an important and physiologically relevant approach to the study of amyloid fibrillogenesis. We have used Quartz Crystal Microbalance with Dissipation (QCM-D) to monitor the changes in layer surface mass density and viscoelastic properties accompanying multi-layer amyloid deposition in situ for the first time. By means of Atomic Force Microscope (AFM) imaging, an unequivocal correlation is established between the interfacial nucleation and growth of glucagon fibrils[2] and the QCM-D response. The combination of the two techniques allows us to study the temporal evolution of the interfacial fibrillation process. We have modelled the QCM-D data using an extension to the Kelvin-Voigt viscoelastic model. Three phases were observed in the fibrillation process: I) A rigid multilayer of glucagon monomers forms and slowly rearranges; II) This multilayer subsequently evolves into a dramatically more viscoelastic layer, containing a polymorphic network of micrometer long fibrils growing from multiple nucleation sites; III) The fibrillar formation effectively stops, due to the depletion of bulk phase monomers, although the process can be continued without a lag phase by subsequent addition of fresh monomers. The robustness of the QCM-D technique, consolidated by complementary AFM studies, should make it possible to combine different components thought to be involved in the plaque formation process and thus build up realistic models of amyloid plaque formation in vitro.

[1]Frokjaer, S. and D.E. Otzen, Protein drug stability: A formulation challange, Nat Rev Drug Discov. 4, 298-306 (2005).

[2]M. Dong, M. B. Hovgaard, S. Xu, D. E Otzen, and F. Besenbacher, AFM Study of Glucagon Fibrillation Via Oligomeric Structures Resulting in Interwoven Fibrils. Nanotech. 17, 4003-4009 (2006).

11:40am **BI-TuM12 Modulating the Orientation and Conformation of Bone Osteopontin and Bone Sialoprotein for Osteoblast Adhesion**, ***M.T. Bernards****, S. Jiang*, University of Washington

Bone tissue is primarily composed of hydroxyapatite (HAP), which accounts for 70-90% of the mass of bone. The remaining 10-30% is composed of protein, of which ~90% has been shown to be collagenous. During natural bone formation cells first lay down a collagenous matrix composed of primarily type 1 collagen. After this collagen network is formed, proteins bind to the matrix and then the matrix is mineralized to form bone. While a number of proteins have been located within bone, only osteopontin (OPN) and bone sialoprotein (BSP) have been localized in the matrix ahead of the mineralization front. Additionally, both of these proteins have been found to be enriched at bone-implant interfaces. These two proteins are hypothesized to play an important role in cellular adhesion at these interfaces. This work details efforts to understand the roles of OPN and BSP in cellular binding to engineered systems mimicking the collagen and mineralized interfaces of bone. In the first part of this work, we compared the cell binding abilities of OPN and BSP when specifically bound to collagen. Both of these proteins have been shown to have a specific binding interaction with collagen and this was confirmed through the development of radiolabeled adsorption isotherms for each of the proteins. These isotherms were used to determine adsorption parameters that resulted in identical amounts of adsorbed protein, to directly compare the cell binding properties of these two proteins when specifically bound to collagen. The results indicate that OPN promotes a greater amount of cell binding to a collagen interface than BSP. The second part of this work compared the cell binding abilities of OPN and BSP when they are specifically bound to HAP, mimicking mineralized bone interfaces. HAP was formed from a simulated body fluid and characterized by scanning electron microscopy, energy dispersive x-ray spectroscopy, and electron spectroscopy for chemical analysis. Both OPN and BSP have been shown to have a specific HAP binding domain and this was confirmed through the development of radiolabeled adsorption isotherms. The cellular adhesion to HAP was then compared with identical amounts of adsorbed proteins. The results of this work indicate that BSP has a more favorable orientation/conformation for cellular adhesion as compared to OPN. However, this influence on cell binding is eliminated when the surface roughness of the underlying HAP substrate becomes too great.

12:00pm **BI-TuM13 Characterization of ECM Protein Production in Spatially Cultured Hippocampal Neurons on Micro-Patterned Surfaces in Serum-Free Conditions**, ***M. Ramalingam****, S. Kootala, N. Bhargava, M. Stancescu, M. Hirsh-Kuchma, M. Klimov, P. Molnar, J.J. Hickman*, University of Central Florida

Spatial positioning of neurons on patterned surfaces and characterization of their functional synaptic connectivity and specific extra-cellular matrix (ECM) protein productivity is of great importance for the fabrication of neuron-based biosensors and in developmental cell biology. We have determined that a combination of traditional biological analysis techniques, such as SDS-PAGE and PT-PCR, and surface analytical techniques, such as X-ray Photoelectron Spectroscopy (XPS) and SIMS, is a good approach for ECM analysis. Here, we report on ECM deposition on patterns of hippocampal neurons on surfaces composed of self-assembled monolayers (SAMs) of two different organic compounds, trimethoxysilylpropl-diethylenetriamine (DETA) and tridecafluoro-1,1,2,2,-tetrahydroctyl-1-tricholorsilane (13F) as well as unpatterned controls, in a serum-free culture condition. The patterns were characterized by XPS, contact angle goniometry, electroless metallization and surface profilometry to confirm their surface composition, wettability and topography. Immunostaining of cultured neurons for synapsin I and microtubule-associated proteins (MAP-2) confirmed the pre- and post-synaptic formation. The electrophysiological study of neurons cultured for 14 days further confirmed the functional synaptic connectivity. The deposition and composition of ECM proteins were determined by immunocytochemistry, confocal laser spectroscopy and reverse transcriptase –polymerase chain reaction (RT-PCR), and it was found that the neurons produce laminin, collagen, fibronectin and vitronectin at differing amounts depending on the conditions. We have

quantified the amounts of these proteins using Western Blot and SIMS spectroscopy. The overall results indicate that the neurons cultured on patterns secrete ECM proteins in a differential fashion and these data will have significant implications in engineering functional neuronal systems and hybrid devices.

Plasmonics Topical Conference

Room: 619 - Session PL+BI-TuM

Plasmon-mediated Sensing and Biosensing

Moderator: J.C. Owrutsky, Naval Research Laboratory

8:00am **PL+BI-TuM1 Combining Surface Plasmons, Enzyme Chemistry and Nanoparticles for Ultrasensitive Biosensing**, ***R.M. Corn***, *H.J. Lee, A.W. Wark*, University of California-Irvine **INVITED**

Surface bioaffinity biosensors have become invaluable biotechnological tools for the rapid, multiplexed detection of biomolecules. In the last decade, a number of surface-sensitive spectroscopic techniques based on changes in the local optical index of refraction near an interface upon adsorption have emerged as attractive alternatives to traditional fluorescence-based detection methods for surface bioaffinity biosensing. For example, we have successfully applied surface plasmon resonance imaging (SPRI) to measure the bioaffinity adsorption of DNA, RNA, antibodies, proteins and biomarkers. In this talk we will describe our recent efforts to create the next generation of ultrasensitive biosensors which use a combination of (i) surface enzyme chemistry and (ii) nanoparticle surface incorporation. For example, SPRI, DNA microarrays, nanoparticles and poly(A) RNA polymerase can be used together for ultrasensitive microRNA profiling measurements at femtomolar concentrations. In addition, we will also describe a new optical technique, nanoparticle-enhanced diffraction gratings (NEDG), that can be used to create novel ultrasensitive biosensors.

8:40am **PL+BI-TuM3 Polarization-Dependent Surface Enhanced Raman Scattering from Silver Nanoparticle Arrays**, ***W. Luo***, *P. Chu, D.L. Mills, R.M. Penner, J.C. Hemminger*, University of California, Irvine

One specially promising method to design Surface Enhanced Raman Scattering (SERS) substrates is to fabricate silver nanoparticle arrays with nanoscale gaps that can carry strong and localized surface plasmon resonances. Studies have revealed that dramatic SERS phenomena require interparticle spacings to be around 10 nm or less which is hard to achieve with current fabrication technologies. We present the straightforward fabrication of ordered spherical silver nanoparticle arrays with gaps less than 10 nm on Highly Oriented Pyrolytic Graphite (HOPG). Physical Vapor Deposition (PVD) of silver on HOPG under controlled experimental conditions results in the self-assembly of rows of silver nanoparticles. Straight rows of particles are obtained that extend over distances as large as 100 microns. Arrays of rows of particles are parallel over similar distances. Since these 2-dimentional particle arrays are organized over 100's of microns they are easily addressed in conventional optical experiments. Using thiophenol adsorbed on the silver particles as probe molecules, highly polarization-dependent enhanced Raman scattering has been observed form these particle arrays. When light is polarized along the axis of the nanoparticle arrays, the enhanced Raman spectra are much stronger than when light is polarized perpendicular to the axis of the nanoparticle arrays. Theoretical calculations of the electromagnetic response of the interacting nanoparticles to a light field will also be presented. Ongoing research with these arrays includes a number of different studies. Among these is the use of the particle arrays as a collection of "nano-electrodes". For example, electrodeposition of semiconductors on the silver nano-arrays has been accomplished with the subsequent observation of enhanced photoluminescence.

9:00am **PL+BI-TuM4 Nanoplasmonic Sensing of Surface and Bulk Modifications of Metallic Nanoparticles using Localized Surface Plasmon Resonances: Studies of Hydrogen Uptake in Supported Pd Nanoparticles and Oxidation of Al Nanoparticles**, ***I. Zorić***, *C. Langhammer, E. Larsson, B. Kasemo*, Chalmers University of Technology, Sweden

We propose a novel nanoplasmonic sensing scheme for optical, real time, monitoring of property changes in metal nanoparticles. The property changes can be both bulk and/or surface changes induced e.g. by interaction with the surrounding medium. To sense these changes we use the localized surface plasmon resonance (LSPR) of the nanoparticle as signal transducer for remote optical readout. The high sensitivity of the LSPR (i.e. plasmon energy and extinction cross-section) to electronic, structural and shape changes, taking place in the nanoparticle, makes the latter an extremely sensitive and non-invasive probe for studies of surface and bulk changes in nanoparticles. Furthermore, since the LSPR seem to be a rather universal feature for nano-confined metallic systems the proposed sensing method is quite universal. The supported metallic nanodisks were fabricated by the hole-mask colloidal lithography method relying on electrostatically self-assembled polystyrene beads as evaporation masks. The method is suitable for fabrication of large-areas covered by nanostructures allowing for easy spectroscopic studies. The proposed sensing scheme was used to study: a) metal hydride formation in nanometer sized Pd disks exposed to hydrogen atmosphere and b) oxidation of Al nanodisks. In both cases we have quantified the optical repsonse by complementing the optical studies with gravimetric studies (QCM-D). In the latter case the same processes were studied by monitoring the frequency and dissipation shifts when Pd or Al nanoparticles, prepared on the of the quartz crystal microbalance electrode, were exposed to the hydrogen and oxidizing environment respectively. In addition, AFM and SEM studies were used to characterize the morphological changes induced during the process of interest. Te most important results include: a) hydrogen pressure-composition isotherms covering the solid solution (α) phase, the coexistence region of the α and hydride (β) phases, and finally the pure hydride phase at large hydrogen pressure. Similarities and differences to the corresponding isotherms for 2D continuous Pt films are also presented. b) Al nanoparticle oxidation kinetics in water shows an initially fast oxide growth followed by a transport limited slower kinetics accompanied by oxide shell cracking. We also show how extension of this sensing approach from nanoparticles to thin films can be made by using LSPR of the 100nm diameter holes made in continuous films.

9:20am **PL+BI-TuM5 Surface Modification of Metallic Nanoparticles for Plasmonics Applications: Potential, Challenges and Advances in the Field**, ***V.H. Perez-Luna***, Illinois Institute of Technology **INVITED**

Nanoparticles of noble metals such as gold and silver exhibit size and shape dependent optical properties that are sensitive to changes in the dielectric environment and degree of aggregation. These properties arise from collective oscillations of plasmons excited by incident light. For metals such as gold and silver resonant excitation of particle plasmons can be tuned to occur in a wide region of the visible and near infrared spectrum by proper manipulation of size and morphology. In addition to their interesting optical properties, excitation of particle plasmons by light give rise to enhanced electric fields in the vicinity of nanoparticles. The enhanced electric fields hold enormous potential for biosensing applications using Surface Enhanced Raman Scattering and Surface Enhanced Emission of Fluorescence. Despite this potential, some applications remain largely unexploited due to inherent morphological instability of anisotropic metallic nanoparticles, irreversible aggregation, and difficult surface modification when templating surfactants such as hexadecyltrimethyl ammonium bromide are used to synthesize anisotropic nanoparticles. Surface modification can overcome these obstacles but has not received sufficient attention. This presentation will focus on understanding displacement reactions at the surface of nanorods and model crystalline gold surfaces; the effect ofsurface modification in overcoming morphological instability; and potential applications in biosensing. Specific applications presented involve the combination polymers and nanoparticles for detection; creation of environmentally sensitive nanostructured surfaces; and photostability of fluorophores in the vicinity of metallic nanorods. New opportunities and future challenges will be discussed.

10:40am **PL+BI-TuM9 Environmental Sensitivities of Localized Surface Plasmon Resonances of Immobilized Nanoparticles: Substrate Modulation of Generic Bulk Phase Results**, *M.M. Miller, S. Chen, A. Chilkoti*, ***A.A. Lazarides***, Duke University

The plasmon bands of metal nanoparticles are known to be sensitive to the refractive index of the environment. The magnitude of the sensitivities vary widely for resonances supported by particles of various shape, size, and composition. Through spectral simulation, however, it has been shown that the resonant frequency of particles suspended in a medium have sensitivities to refractive index of the medium that are determined with high accuracy by the plasmon frequency and the dielectric properties of the particle and medium, when the particle is composed of a single component and of modest phase volume.[1],[2] Immobilized particles, in contrast, display sensitivities to the exchangeable component of their media that are reduced relative to those of suspended particles and expected to be dependent upon the nature and localization of the mode and its interaction with the substrate. Here, we report measurements and simulations of the refractive index sensitivities of a family of gold nanorods immobilized on glass, and a comparison of the immobilized particle sensitivities with the generic sensitivities of plasmons supported by suspended particles. The refractive index sensitivities of the immobilized rods are found to be well predicted by

a band location dependent sensitivity function reduced from the generic solution phase sensitivity function by a constant scale factor derived from comparison of simulation and theory. The applicability of the result to immobilized particles of other shapes, sizes, and compositions will be discussed within the framework of sensitivity theory.

[1] Miller, M. M.; Lazarides, A. A. "Sensitivity of Metal Nanoparticle Surface Plasmon Resonance to the Dielectric Environment" J. Phys. Chem. B 2005, 109, 21556-21565

[2] Miller, M. M.; Lazarides, A. A. "Sensitivity of Metal Nanoparticle Plasmon Resonance Band Position to the Dielectric Environment as Observed in Scattering" J. Opt. A: Pure Appl. Opt. 2006, 8, S239-S249.

11:00am **PL+BI-TuM10 Controlled Plasmonic Coupling in Reconfigurable Nanoparticle Assemblies**, ***D.S. Sebba***, *T.H. LaBean, A.A. Lazarides*, Duke University

Metal nanoparticles (MNPs) support localized surface plasmon resonances that are sensitive to particle shape, size, composition, and the presence of other polarizable particles and materials. Advances in MNP synthesis and surface chemistry have yielded biomolecule nanoparticle conjugates that interact specifically with oligonucleotides, peptides, and proteins. The specific recognition properties of these components have been exploited in responsive plasmonic systems with formats that range from amorphous solution phase particle networks to immobilized colloidal monolayers and individually responsive particles. For molecular detection applications, various formats have various advantages, with single particle sensors generally offering ease of control and multi-particle systems offering strong plasmon modulation. Here, we report plasmon modulation in pre-formed, few particle assemblies linked by reconfigurable DNA nanostructures. The investigation is motivated by the potential of reconfigurable few particle assemblies to provide control of plasmon coupling in a format that displays high responsiveness per molecule. In the coupled system upon which we report, DNA nanostructures tether satellite MNPs to a core MNP. The DNA nanostructures use duplex DNA to control interparticle separation and are responsive to target strands that modulate interparticle helix length. The reconfigurable assemblies are characterized in two states, using dynamic light scattering and transmission electron microscopy to monitor structure and scattering spectroscopy to monitor plasmonic properties. A two state structural model is tested by comparison of spectroscopic data with spectra calculated for structures defined by core/satellite stoichiometry from TEM and interparticle separations from measurements in DNA-linked networks. Thermodynamic properties derived from melting transition data collected from the reconfigurable DNA linker ex-situ are reported and reviewed as a source of insight into DNA nanostructure control of system stability.

11:20am **PL+BI-TuM11 Metal Films with Arrays of Tiny Holes: Infrared Plasmonic Scaffolding for Spectroscopy**, ***J.V. Coe***, *K.R. Rodriguez, S. Teeters-Kennedy, H. Tian, J.M. Heer*, The Ohio State University **INVITED**

The surface plasmon (SP) mediated, extraordinary transmission of metal arrays of subwavelength holes has been moved into the infrared (IR) region in order to overlap with the traditional range of molecular vibrations. SP-enhanced IR absorption spectra are recorded (using standard FTIR instrumentation) of metal-supported self-assembled alkanethiol monolayers, phospholipid bilayers, gramicidin (an antibiotic peptide) and cholesterol in phospholipid bilayers, as well as hexadecane thin films. The interaction of a SP resonance and a vibrational excited state has been examined by tuning a SP resonance (both by film thickness and angle of the mesh) through the primary rocking vibration of the hexadecane molecule producing vibrational band intensity changes, peak shifts, and lineshape changes. The nature of the enhancements will be discussed.

12:00pm **PL+BI-TuM13 Photo-Recognition and Control of a Small Number of Moleclues at Metal Nano-Gap Arrayed on Solid Surface**, ***K. Murakoshi***, Hokkaido University, Japan

Detection, recognition, and control of single molecules are a common theme in recent advanced technologies. It has been demonstrated that the surface-enhanced Raman scattering (SERS) phenomenon can drastically increase the scattering cross section, which is comparable to that of fluorescence at high quantum yield. Although the importance of single-molecule SERS (SM-SERS) has been well recognized from the early stage of its discovery, there are only a few examples supporting the observation of SM-SERS. In the present study, well-ordered, periodic metal nano-dot dimer arrays were prepared. The gap distance between two metal dots was optimized to show intense SERS in an aqueous solution. The system was also applied to control the adsorption of target molecules. In-situ Raman spectroscopic measurements with 785 nm excitation were carried out in aqueous solution using metal nano-gap array. Intense Raman signals were observed when the metal dimer structure was optimized. The SERS activity was dependent upon on the structure of the metal dimer with a distinct gap distance, suggesting that the intense SERS originates from the gap part of the dimer. Characteristic time-dependent spectral changes were observed both in Stokes and anti-Stokes region. In the system of Au dots array, relatively stable SERS signals was observed even under relatively strong photo-irradiation. Possibility of the molecular manipulation by electromagnetic filed will be discussed based on the characteristic behavior of SERS signals observed under relatively strong photo-irradiation.

Biomaterial Interfaces

Room: 609 - Session BI-TuA

Engineered Cellular Interfaces

Moderator: H.E. Canavan, University of New Mexico

1:40pm **BI-TuA1 Simultaneous Deposition of Endothelial Cells and Biomaterials for Human Microvasculature Fabrication**, *X. Cui, P.V. Kreuk, **T. Boland***, Clemson University

The current tissue engineering paradigm is that successfully engineered thick tissues must include vasculature. As biological approaches alone such as VGEF have fallen short of their promises, one may look for an engineering approach to build microvasculature. Layer-by-layer approach for customized fabrication of cell/scaffold constructs have shown some potential in building complex 3D structures. With the advent of cell printing, one may be able to build precise human microvasculature with suitable bioink. Human Microvascular Endothelial Cells (HMEC) and fibrin were studied as bioink for microvasculature construction. Endothelial cells are the only cells to compose the human capillaries and also the major cells of blood vessel intima layer. Fibrin has been already widely recognized as tissue engineering scaffold for vasculature and other cells, including skeleton/smooth muscle cells and chondrocytes. In our study, we precisely fabricated micron-sized fibrin channels using a drop-on-demand polymerization. This printing technique uses aqueous processes that have been shown to induce little, if any, damage to cells. When printing HMEC cells in conjunction with the fibrin, we found the cells aligned themselves inside the channels and proliferated to form confluent linings. Current studies to characterize the biology and functionality of these engineered microvascular structures will be presented. The preliminary data suggests that a combined simultaneous cell and scaffold printing can promote HMEC proliferation and microvasculature formation.

2:00pm **BI-TuA2 Cells, Surfaces, Spaces and Forces: What makes a tissue?**, ***K.D. Hauch**, D.J. Mortisen, M.A. Laflamme, C.E. Murry, B.D. Ratner*, University of Washington **INVITED**

Tissue engineering strives to combine parenchymal and other cells with porous biomaterial scaffolds; to grow tissue like constructs that can be used to repair diseased or damaged tissues and organs. The natural course of development, as well as some (but not all) processes of wound repair and regeneration, depend upon complex parameters including: the changing composition and capabilities of the cells that populate the tissue; molecular cues from the interface between cell and its environs; the structural space wherein the cells reside; and mechanical forces. All these and more act to guide the processes that results in a hierarchical living tissue with appropriate structure and function. Here we explore these issues in the context of cardiac tissue engineering. Adult cardiomyocytes demonstrate little if any proliferative potential. However, using an appropriate schema of soluble cues, large quantities of proliferating cardiomyocytes as well endothelial cells can be derived from cultures of human embryonic stem cells, to be used for tissue engineering. Postulating the importance of scaffold geometry, novel scaffolds were constructed with appropriately sized spaces and shapes, providing an engineered support structure that mimics aspects of native muscle architecture. Molecular cues are provided by immobilizing adhesion proteins on the scaffold and delivering other soluble factors to stimulate cell survival, proliferation, and ultimately vascularization. huESC-derived cardiomyocytes populate these scaffolds and survive at high cell densities in culture. Finally, the application of cyclical mechanical stress during in vitro culture is seen to enhance cardiomyocyte size, survival and functional organization. The analysis of these engineered tissues depends on both standard immunohistochemical observations, as well as newer visualization tools, including Digital Volumetric Imaging, a microscopic 3D serial sectioning and reconstruction technique. Together, the appropriate application of proliferative cardiomyocytes to carefully engineered scaffolds featuring spaces of appropriate size and shape, in conjunction with soluble and mechanical cues can lead to the development of a robust functional unit of cardiac muscle.

2:40pm **BI-TuA4 Expanding Human Embryonic Stem Cells without Feeder Cells on Chitosan-Alginate 3D Porous Scaffolds**, ***M.C. Leung**, L. Zhensheng, M. Zhang*, University of Washington

The tremendous interest in human embryonic stem (hES) cells is motivated by a wide range of potential therapeutic, diagnostic, and fundamental research applications. To preserve their undifferentiated state, two-dimensional co-culture with feeder cells is standard practice.[1,2,3] In order to develop therapeutic applications, a system for the undifferentiated expansion of hES cells in pure culture must be developed to prevent xenogenic contamination.[4,5,6] With BG01V cells as a model, porous chitosan-alginate (CA) scaffolds were studied as a three dimensional (3D) substrate for undifferentiated hES cell proliferation. It was observed that hES cells attached, proliferated, expressed relevant transcription factors, translated appropriate markers, and retained pluripotency after 21 days of cultivation. Furthermore, the 3D CA culture system replicates the structure of natural extra cellular matrix, creating additional opportunities for regenerative medicine. This method realizes the goal of expanding pure hES cell populations in vitro while preserving undifferentiated state, and represents a significant advancement in hES cultivation technique.

[1]Choo, A., Padmanabhan, J., Chin, A., Fong, W. J. & Oh, S. K. Immortalized feeders for the scale-up of human embryonic stem cells in feeder and feeder-free conditions. J Biotechnol 122, 130-41 (2006).

[2]Oh, S. K. et al. High density cultures of embryonic stem cells. Biotechnol Bioeng 91, 523-33 (2005).

[3]Richards, M., Fong, C. Y., Tan, S., Chan, W. K. & Bongso, A. An efficient and safe xeno-free cryopreservation method for the storage of human embryonic stem cells. Stem Cells 22, 779-89 (2004).

[4]Stacey, G. N. et al. The development of 'feeder' cells for the preparation of clinical grade hES cell lines: challenges and solutions. J Biotechnol 125, 583-8 (2006).

[5]Amit, M. & al., E. Feeder Layer- and Serum-Free Culture of Human Embryonic Stem Cells. Biol Reprod 70, 837-845 (2004).

[6]Hoffman, L. M. & Carpenter, M. K. Characterization and culture of human embryonic stem cells. Nat Biotechnol 23, 699-708 (2005). .

3:00pm **BI-TuA5 Stem Cell Adhesion and Proliferation Correlated with Surface Properties of Copolymer Libraries Synthesised as Micro Arrays**, *A.J. Urquhart, M. Taylor,* University of Nottingham, UK*, D.G. Anderson, R. Langer,* Massachusetts Institute of Technology*, M.R. Alexander, **M.C. Davies***, University of Nottingham, UK

In the field of tissue engineering, the search is on for the optimum polymer scaffold material to support the adhesion and proliferation of stem cells for organ regeneration. To accelerate this process, Anderson et al., developed a high throughput screening methodology for the assessment of stem cell interactions with a large combinatorial library of over 500 copolymers1. Initial cellular behaviour with these materials will be driven by surface-cell interactions but until very recently, there was no rapid method of measuring the surface chemistry of such spatially patterned arrays. We report on the first high-throughput screening of the surface chemistry (ToF-SIMS and XPS) and wettability (contact angle, surface energetics) of large copolymer library array spatially patterned as 300 micron islands and polymerized in-situ on a single poly(HEMA) slides. The copolymer library is designed to exhibit a range of surface phenomena and their ability to support the growth of cells (eg, endothelial stem cells, bacteria) was assessed. Statistical analysis of the large surface and biological data sets reveals important relationships linking surface properties and cell interactions that point to the key surface phenomenon that could lead to the development of optimised copolymer surfaces for the development of polymeric scaffolds.

4:00pm **BI-TuA8 Hierarchical Control of Form and Function in the Heart**, ***K.K. Parker***, Harvard University **INVITED**

Expression of sarcomeric proteins is necessary, but not sufficient, for contraction of cardiac myocytes. Posttranslational processes contribute to regulation of muscle growth during cardiac development, normal function, and disease. However, little is known about the mechanisms and signals that potentiate directional muscle growth and the self-assembly and organization of sarcomeres, myofibrils, cells, and tissues. These structures appear to be optimized for their contractile function. In order to elucidate the structure-function relationships that govern contractility, we have developed computational and experimental models of self-assembly and organization in cardiac myocytes in vitro. By controlling only 2D boundary conditions imposed on the myocyte, we are able to engineer predictable myofibrillar patterns and contractility of individual myocytes. These experiments have revealed how the extracellular matrix provides an important set of instructions for self-assembly of the myocyte cytoskeleton architecture which serves as a template for myofibrillar patterning. Our results suggest the post-translational mechanisms that regulate cardiac organo- and pathogenesis.

4:40pm **BI-TuA10 BioArtificial Matrices to Control Blood Vessel Network Formation**, ***E.A. Phelps**, A.J. García*, Georgia Institute of Technology

Vascularization of engineered regenerative constructs is a major obstacle in the development of clinically significant regenerative medicine. The ability of regenerative constructs to recapitulate normal blood vessel wiring is central to their successful integration with host tissue, proper physiological function, and long term survival. The natural formation of new blood vessel

networks is driven by spatially and temporally controlled presentation of positive and negative cues that direct cell behavior to initiate vessel sprouting, migration, and stabilization.[1] We have developed a strategy for engineering regenerative constructs with spatially patterned biomolecules to direct the formation of orderly networks of blood vessels in artificial biomaterials. Our approach employs a photopatterning technique to convalently link bioactive peptides to poly(ethylene glycol) (PEG) hydrogels to modulate and direct cell function. In this system, peptides are attached to the surface of PEG hydrogel through the use of a photoactive crosslinking agent. Peptides are patterned on the hydrodel by exposing the peptide and crosslinker solution on the surface to UV light through a Mylar photomask. We achieved sharply defined patterns of fluorescently labeled peptide with 10 µm features. We anticipate that the system can easily produce higher resolution patterns. We employed the photopatterning technique to create various patterns of the adhesive ligand RGDS on a nonadhesive PEG background. We have shown that we can constrain the adhesion and morphology of NIH fibroblast cells to the patterned RGDS with this system. We have also used RGDS functionalized PEG hydrogels to induce tubule formation of human aortic endothelial cells, and we have successfully created patterns of labeled RGDS resembling branching microvasculature. We plan to use these patterns to direct the growth of vascular sprouts from explanted sections of mouse aorta into a vascular network. Ultimately we will employ a system to pattern ligands in 3D to direct vascularization of an implanted hydrogel in vivo. The central hypothesis of this work is that spatiotemporal presentation of bioactive cues will result in directed vascularization of engineered hydrogels from the host tissue and that increased vascularization will result in improved healing, integration, and function of regenerative constructs.

[1]M. P. Lutolf and J. A. Hubbell, Nature biotechnology 23 (1), 47 (2005).

5:00pm **BI-TuA11 Cytoskeleton Structure and Focal Contact Points on a Micro 3D Patterned Film**, ***H. Sunami**, E. Ito,* Hokkaido University, Japan*, M. Tanaka,* Tohoku University, Japan*, S. Yamamoto,* Hokkaido University, Japan*, M. Simomura*, Tohoku University, Japan

Micro fabrication of cell culture substrates is one of the most significant subjects in the field of biomaterial research. Recently we found that endothelial cells can proliferate rapidly on a micro 3D patterned film (honeycomb film). The cell shape and cytoskeleton structure on the honeycomb films were clearly different from those on a flat film. In order to elucidate the effect of honeycomb films as a 3D scaffold for cell culture, it is needed that the 3D observation of cell behaviors such as the morphological change, expression of cytoskeleton, expression of contact points on extracellular adhesion molecules, and migration on the honeycomb films during cell culture. In this research, effects of 3D honeycomb pattern on above cell behaviors were observed.

Biomaterial Interfaces

Room: 4C - Session BI-TuP

Biomaterials Interfaces Poster Session

BI-TuP1 Investigating the Adhesion of Biomolecules on Plasma Polymerized Thin Films, ***R. Foerch***, *S. Brueninghoff, E.-K. Sinner, W. Knoll, R. Berger*, Max-Planck-Institut for Polymer Research, Germany

The controlled adhesion of biomolecules on surfaces has become a major topic of interest in biomaterial surface design. The interest in this has been triggered by a broad range of biomaterial applications. For example, those that rely on proteins and cells immobilized on solid surfaces for the development of new implant materials and tissue regeneration. It is generally accepted that both the surface morphology and the surface chemistry need to be in concert to ensure optimum conditions for biomaterial adhesion. However, the surface interactions are very complex and are not completely understood. Further, there are only very few analytical tools available that deliver reliable, real time insights into the biomaterial/ surface interactions. We present recent results in which plasma polymerization techniques have been used to prepare "model" surfaces that can be conditioned towards the adhesion of a particular mammalian cell line (P19 progenitor cells). Techniques such as Surface Plasmon Resonance (SPR), Micro-Cantilever Sensor technology (MCS) and standard optical microscopy have been used to study in real time the adsorption of proteins and P19 cells on such surfaces. Correlations to the chemical environment at the interface are made.

BI-TuP2 Tailoring Surface Properties of Spider Silk Protein Films for Biomaterial Applications, ***P.A. Johnson***, *H. Zhang, C. Skinner, T.G. Martinez-Servantez*, University of Wyoming

Spider silk is well known for its unique, outstanding material properties. Dragline spider silk in particular is one of the strongest natural materials with a high degree of elasticity. These properties, together with its inherent biodegradability and biocompatibility, make it a promising biomaterial for tissue engineering applications. Though the best material properties are derived from native dragline spider silk, producing spider silk naturally is not practical to generate sufficient quantities for biomedical applications. Recombinant spider silk proteins have been successfully produced in bacterial expression systems as well as in goat's milk. In our studies we compared the surface properties and biological responses of native major ampullate silk from N. clavipes, the major ampullate proteins produced in E. coli, and the major ampullate proteins produced in goats' milk. Thin films were cast from hexafluoroisopropanol and then treated with 90% methanol. The films with and without methanol treatment were characterized by AFM, SEM and contact angle analysis. The untreated films initially had very different surface properties, but after methanol treatment the contact angles and surface roughness converged to similar values. Once processes for generating consistent films were established, the biological response of the films was determined. Protein adsorption studies were conducted via mass sensitive techniques (QCM-D). Cellular responses were established to determine cell adhesion, cell morphology and cell proliferation. The films were protein resistant and inhibited cell adhesion. Therefore, to promote cell attachment and growth the spider silk films were modified with cell binding peptides.

BI-TuP3 Self-Assembly of Biomolecules at Surfaces, Characterized by NEXAFS, ***X. Liu***, *F. Zheng,* University of Wisconsin-Madison, *A. Jürgensen,* Canadian Synchrotron Radiation Facility, Synchrotron Radiation Center, *V. Perez-Dieste,* Universitat Autònoma de Barcelona, Spain, *D.Y. Petrovykh,* University of Maryland and Naval Research Laboratory, *N.L. Abbott, F.J. Himpsel*, University of Wisconsin-Madison

Surface science has made great strides towards tailoring surface properties via self-assembly organic molecules. It is now possible to functionalize surfaces with complex biomolecules, such as DNA and proteins. However, probes of the chemical orbitals involved in this process have remained scarce. Here we show how NEXAFS (Near Edge X-ray Absorption Fine Structure spectroscopy) can be used to characterize the assembly of biological molecules at surfaces in atom- and orbital-specific fashion. The technique is illustrated by self-assembled monolayers with customized terminal groups. Applications are demonstrated by DNA oligonucleotides and Ribonuclease A[1], a small protein containing 124 amino acids. The N 1s absorption edge is particularly useful for characterizing DNA and proteins, because it selectively interrogates the π^* orbitals in nucleobases and the peptide bonds in proteins. Information about the orientation of molecular orbitals is obtained from the polarization dependence. Quantitative NEXAFS models are developed to explain the polarization dependence in terms of molecular orientation and structure.

[1]Xiaosong Liu et al., Langmuir 22, 7719 (2006).

BI-TuP4 Adsorption of an S-layer Bacterial Protein by Total Internal Reflection UV Absorption Spectroscopy, ***M.A. Bratescu,*** Nagoya University, Japan, *D.B. Allred,* Nagoya University, Japan, and University of Washington, *N. Saito,* Nagoya University, Japan, *M. Sarikaya,* University of Washington, *O. Takai*, Nagoya University, Japan

Surface-layer (S-layer) proteins from many species of bacteria and archaea self-assemble into two-dimensional supramolecular arrays and form specific space groups. Although S-layer proteins are now used as molecular and nanoscale templates for nano- and bio-nanotechnology, the fundamental bases of assembly and ordered organization are still under study. The purpose of our research is to characterize adsorption specificity, surface interactions, and assembly of S-layer proteins on solid surfaces. We use absorption of UV evanescent light produced by multiple total internal reflections in a quartz IRE sensor which is sensitive to a depth of a few tens of nanometers. The S-layer proteins were prepared as described in Ref. [1], starting from a cell culture of Deinococcus radiodurans. A final concentration of protein in sodium dodecyl sulfate was estimated to be 0.5 mg mL $^{-1}$. For experiments, the protein solution was diluted in deionized water to a concentration of 0.2 mg mL $^{-1}$. A comparative analysis of the S-layer protein adsorption was performed on different functionalized surfaces (amino- and hydroxyl- terminated) or surfaces deposited with noble metals (platinum and gold). The amino-terminated monolayer was obtained by dipping freshly cleaned quartz into a 1 wt % solution of (3-aminopropyl) trimethoxysilane in toluene for 3 hours at 60 ° C. The hydroxyl-terminated surface was obtained by photochemical exposure of quartz to VUV radiation at 172 nm. The noble metals were deposited by magnetron sputtering. S-layer protein absorption bands were found at 274 nm and 307 nm, characteristic of tryptophan and tyrosine residues, which have UV absorption bands in the spectral region 270 - 350 nm. The strongest absorption was obtained when S-layer proteins were adsorbed on an amino-terminated surface. Time dependence of protein adsorption will be correlated with data obtained by atomic force microscopy analysis of S-layer proteins on different solid surfaces to derive a better understanding of the adsorption process.

[1]D.B. Allred, M. Sarikaya, F. Baneyx, D.T. Schwartz, Nano Lett., 5 (2005) 609.

BI-TuP5 Adsorption Kinetics and Dynamics of Fibrinogen with Surface Interactions, ***N. Saito***, *Y. Sato, O. Takai*, Nagoya University, Japan

The control of blood coagulation on biomaterial surfaces is an urgent issue in the medical field. The main process occurring in blood coagulation is the polymerization of fibrinogen into erythrocyte-trapping fibrin. Other plasma proteins are also involved in initiating this process. The multi-interactions among proteins and material surface govern the process. The molecular structure of fibrinogen as a free molecule has been investigated in detail. However, its adsorption state on a biomaterial surface has yet to be elucidated. Recently, the adsorption state of fibrinogen on various substrates has been the subject of intensive investigation employing atomic force microscopy (AFM). Such use of AFM has been crucial in the study of molecular biology on surfaces. In this study, the adsorption kinetics and dynamics of fibrinogen with the interaction of heparin onto hydrophobic and hydrophilic surfaces is investigated with AFM analysis and QCM (quartz crystal microbalance). CH3-terminated self-assembled monolayer (SAM) and SiOH-terminated substrates were utilized as the hydrophobic and hydrophilic surfaces. Fibrinogen adsorption proceeded following Langmuir type. On the process, the orientation of fibrinogen on the sample surface was changed. Heparin did not promote desorption of fibrinogen but the adsorption on the hydrophilic surface though it is well known as an anti-coagulation factor. On the other hands, heparin promoted the adsorption on the hydrophobic surface. The differences were discussed based on 3-body interactions analysis.

BI-TuP6 In situ Analysis of Fibrinogen on Self-Assembled Monolayers by Evanescent Optical Spectroscopy, ***M. Matsuno***, *T. Ishizaki, O. Takai, N. Saito*, Nagoya University, Japan

Fibrinogen is one of proteins in blood plasma and plays a great important role on blood coagulation. Many researchers have investigated the adsorption of fibrinogen on various substrates in order to develop inactive biomaterials for fibrinogen. However, the coagulation mechanism has not been understood yet, since it is due to interactions among many factors, ex. other proteins and ions. The research on fibrinogen adsorption from molecular viewpoints has been required. In this study, we aimed to

understand fibrinogen adsorption on OH-terminated, CH_3-terminated, NH_2-terminated and poly(ethylene glycol) (PEG) surfaces using an evanescent optical spectroscopy, an atomic force microscopy (AFM) and a zeta potentiometry. The evanescent optical spectroscopy allows us to detect few fibrinogens on surfaces with high-sensitivity. A quartz glass was used as a substrate, which worked as an optical waveguide. A vacuum ultraviolet (VUV) lamp with a wavelength of 172 nm irradiated the quartz substrates. The surface changed to OH-terminated surface. CH_3-terminated surface was prepared from n-octadecyltrichlorosilane (OTS) dissolved in toluene through liquid phase method. NH_2-terminated and PEG surfaces were prepared from n-(6-aminohexyl)aminopropyltrimethoxysilane (AHAPS) and 2-methoxy[polyethyleneoxy]propyltrimethoxysilane (MPEOPS) through a vapor phase method. Dried fibrinogen was dissolved in phosphate buffered saline (PBS). The concentrations of fibrinogen were adjusted to 0.1, 1 and 10μM. Fibrinogen adsorption process was monitored by the evanescent optical spectroscopy. An absorption peak was observed at wavelength of ca. 280nm, which is attributed to tyrosine and tryptophan in fibrinogen. The change of the intensity against time was determined by types of surfaces. In order to reveal the difference, the fibrinogens on the surfaces were observed by AFM. In addition, zeta potentials of the fibrinogen and the sample surfaces were obtained in order to elucidate the effect of electrostatic interaction among them on adsorption. Finally, we propose a kinetic model of the adsorption.

BI-TuP7 Determining the Surface Release Kinetics of KGF Protein from a Biodegradable Polymer Film, ***S.A. Burns***, *J.A. Gardella Jr.*, SUNY Buffalo

Biodegradable polymers are of interest in developing strategies to control protein drug delivery. The protein that was used in this study is Keratinocyte Growth Factor (KGF) which is a protein involved in the re-epithelialization process. The protein is stabilized in the biodegradable polymer matrix during formulation and over the course of polymer degradation with the use of an ionic surfactant Aerosol-OT (AOT) which will encapsulate the protein in an aqueous environment. The release kinetics of the protein from the surface of the polymer matrix requires precise timing which is a crucial factor in the efficacy of this drug delivery system. Determining the release kinetics was accomplished by a two fold method. The first step was to measure the accumulation of the surfactant and protein at the surface of the polymer film. X-ray Photoelectron Spectroscopy (XPS) was used to measure the surface concentration of the surfactant and polymer using the unique elemental composition of these compounds. The surfactant has been identified from the polymer matrix using the sulfur region while protein identification utilizes the nitrogen signal. Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) was used in the same capacity to identify the molecular ion peak of the surfactant and polymer and use this to determine surface concentration. The surfactant molecular ion peak was observed in the positive and negative mode at m/z 467 and 422, respectively. These peaks were determined to be [AOT + Na+]+ and [AOT-Na+]-. These methods are used to identify the surfactant and protein from the polymer matrix and are used to measure the rate of surface accumulation. The second step was to compare this accumulation rate with the release rate of the protein into an aqueous solution during the degradation of the biodegradable film. This rate is compared to fluorescence spectroscopy measurements that were done using the autofluorescence of the protein that has been released into an aqueous solution. This study was done to determine the release kinetics of an unmodified biodegradable system containing only a polymer, surfactant, and protein. One method that is currently being used to tune the release rate of the polymer is micro-patterning. The pattern will determine the rate at which the polymer degrades and the rate at which protein is released.

BI-TuP8 Protein Encapsulation in Organo-Functionalized Mesostructured Silica and Titania, *C.T. Burns, S.Y. Choi,* ***M.A. Firestone***, Argonne National Laboratory

Interfacing biomolecules to inorganic frameworks is essential for fabricating robust, functionally integrated biocomposites that may prove useful in a wide range of technologies including biocatalysis, biosensors or protein-based devices. Our work is directed at developing means to integrate biomolecules (e.g., proteins) into 'active' mesostructured inorganics. These active frameworks serve to both improve the mechanical stability (robustness) of the proteins and to facilitate communication with the encapsulated guests. Toward that end, we have synthesized and characterized a variety of photoactive mesoporous silicas and have examined the encapsulation of soluble proteins within them. Specifically, we have both carried out post-synthesis modification of the silica pore surface and prepared organosilicas in which spiropyran groups are homogeneously incorporated throughout the walls of the mesoporous silicas as a means to introduce photo- and redox active moieties within the frameworks. Protein incorporation within mesostructured titania thin films has also been achieved. The synthetic strategies used to prepare these materials, and details of the characterization of the frameworks and the biocomposites will be presented.

BI-TuP9 Dynamic Visible Spectroscopic Ellipsometry Studies of Protein Adsorption and Conformational Change, ***S. Sarkar***, *L. Castro, D.W. Thompson, A. Subramanian, J.A. Woollam*, University of Nebraska, Lincoln

Protein adsorption onto surfaces continues to be widely researched, as it is an everyday occurrence either improving or impeding the quality of life. Often investigated are kinetics of adsorption. In the present work we monitored protein adsorption to surfaces with different chemistries. Modified chitosan surfaces and silicon wafers were used. Spin-cast Chitosan was cross-linked and activated with diepoxides and selected chemical ligands (n-butyl amine, t-butyl amine, n-octyl amine, 2,4,6 Tris and 2-t-Ethyl(butyl amine)) and anti-human albumin molecule. These surfaces were created to preferentially adhere to human serum albumin (HSA). Protein solutions consisting of HSA, immunoglobulin and fibrinogen dissolved in phosphate buffered saline were used to study adsorption processes. Solutions were introduced onto modified chitosan using a fluid cell, and dynamic data optically modeled to obtain protein adsorption profiles. Chitosan is a well known hydro-gel, and spun cast chitosan is anisotropic. Our EMA-based optical model accounts for this anisotropy. A thermodynamic adsorption model was formulated which accounts for changes in protein surface binding capacity during dynamic conformational changes. The model assumes irreversible binding of proteins and a process that is not diffusion limited. The adsorption profiles were then modeled and the parameters compared. Our studies indicate typical protein adsorption processes differ from a simplistic Langmuir model. It also describes changes in protein binding rates during a given adsorption cycle. We propose these regions of data result from surface bound protein conformation changes.

BI-TuP10 Performance and Properties of Poly(N-isopropylacrylamide) Based Switchable Coatings, ***M.A. Cole***, University of South Australia, *H. Thissen,* CSIRO Molecular and Health Technologies, Australia, *N.H. Voelcker,* Flinders University, Australia, *H.J. Griesser*, University of South Australia

Surface modification of biomedical and biotechnological devices using thin polymeric coatings is a popular method employed to alter the interactions of synthetic materials with biomolecules and cells from surrounding biological media and environments. Advancements in this field have been made largely from an interdisciplinary approach combining surface modification and polymer science with biomaterials science and biological studies. Recent research into stimuli-responsive or switchable materials has focused on means to control protein-material and cell-material interactions with respect to directing the spatial location, temporal location and biological function of biomolecules. Controlling the interfacial interactions of biological components is of interest for a wide range of biomedical/biotechnological applications including microarrays, biosensors, drug delivery, cell sheet engineering and 'lab on a chip' devices. As part of ongoing research we report our findings on the grafting and properties of stimuli-responsive coatings incorporating poly(N-isopropylacrylamide) (pNIPAM). Thin films of pNIPAM were prepared via 'grafting to' and 'grafting from' techniques and investigated at temperatures above and below the lower critical solution temperature (LCST) of approximately 32 °C. Switching of coatings between hydrated and collapsed states below and above the LCST was investigated with respect to the degree of change in phase transition and the ability to induce protein adsorption. Surface analysis was carried out using X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and time of flight secondary ion mass spectrometry (ToF SIMS). Adsorption of model proteins, lysozyme and bovine serum albumin were investigated using a quartz crystal microbalance (QCM), optical waveguide lightmode spectroscopy (OWLS), TOF SIMS, and AFM. Results from switching and protein adsorption experiments show the transformation of pNIPAM coatings between low fouling (protein resistant) and fouling (protein adsorbent) states. Colloid probe force analysis of pNIPAM coatings reveal considerable changes in protein-pNIPAM interactions at different temperatures. The present study is expected to assist the development of switchable coatings for biomedical and biotechnological applications.

BI-TuP11 Molecular Dynamics Parameterization for Electrostatic Interactions between Proteins and Biomaterial Surfaces, ***G. Collier***, Clemson University, *B.R. Brooks,* National Institutes of Health, *S.J. Stuart, R.A. Latour*, Clemson University

Since protein-biomaterial interactions govern the biocompatibility of implanted materials, controlling biocompatibility through material design must begin with the study of protein-biomaterial interactions at the atomic level. All-atom molecular dynamics simulation provides an excellent approach to investigate this type of problem. However, current molecular

dynamics simulation methods and parameters are generally not designed to accommodate the unique types of atomic interactions that exist for the case of a protein interacting with a functionalized surface. To address this problem, we have begun adapting the molecular modeling community's range of tools to develop a set that is specifically designed for the simulation of the adsorption behavior of proteins to functionalized surfaces. Protein adsorption behavior is predominantly governed by nonbonded interactions, with electrostatic effects representing the strongest type of these interactions and the type that is most difficult to accurately represent. In an effort to establish the most appropriate method of treating electrostatic interactions for the simulation of adsorption processes, we are evaluating the calculated differences in ion distribution over a charged surface using a variety of nonbonded interaction techniques. Our 4.5 x 4.3 x 10.0 nm^3 model system is comprised of a 150 mM NaCl aqueous solution with TIP3P water over a 50% deprotonated COOH-SAM surface (pK_a = 7.4) with Na^+ counterions. Nanosecond-scale molecular dynamics simulations are then conducted to model the structure of the electric double layer over the surface using a series of different methods to represent the electrostatic interactions of the system, including particle-mesh Ewald, radial cutoffs, isotropic periodic sum, and anisotropic periodic sum methods. The results of these simulations are then compared to the analytical solution of the ion distribution based on the Poisson-Boltzmann equation to gauge the accuracy of each of the different simulation methods. Within each different method, the parameters defining the limits of atomic interactions, such as interaction cutoff distances in the case of radial cutoffs, have been varied to establish a balance between computational cost and simulation accuracy. The results of this study will establish the most efficient and accurate method for the representation of nonbonded electrostatic interactions for the simulation of protein-surface interactions.

BI-TuP12 Photogenerated Surfaces for Carbohydrate-Protein Interactions*, **O. Ramstrom**, Royal Institute of Technology, Sweden, M. Yan*, Portland State University

Photogenerated bioactive surfaces have been developed following a perfluorophenylazide-based, double ligation strategy. Gold-plated quartz crystal microbalance crystals were initially coated with different polymers, either covalently or non-covalently, and the resulting surfaces were subsequently used as substrates for photoinsertion of active photoprobes. A range of different polymers were evaluated, where high biocompatibility and durability could be shown. The photoprobe insertion yielded a range of different carbohydrate-based recognition motifs presented at the surfaces, which were subsequently used as sensor recognition elements in a flow-through quartz crystal microbalance instrumentation. A series of different carbohydrate-binding proteins (lectins) were analyzed, and selectivity and affinity of protein binding could be efficiently monitored. The results clearly show the predicted protein selectivities, demonstrating the applicability of the approach.

BI-TuP13 Microlens Array Patterning of Glass and Silicon for Protein Bioarray Formation*, M.R. Linford, M.C. Asplund, R. Gates, F. Zhang, **G. Saini***, Brigham Young University

Microlens arrays (MLAs) are powerful tools for surface modification. These commercially available optics offer a wide range of shapes and packings for their microlenses. Here we describe the use of MLAs for patterning monolayer-coated glass and silicon substrates. In this process, a nanosecond pulse of laser light is directed through a MLA. In the region where the microlenses focus the light onto the surface the protective monolayer is removed leaving a pattern of spots. A wide variety of proteins, including ferritin and ferritin analogs, adsorb directly onto these spots. A reactive polymer will also adsorb to these spots and undergo subsequent reactions that are typical of bioconjugate chemistry. This process is further demonstrated with 266 nm light on glass, where glass is the substrate material of choice for bioarrays.

BI-TuP14 Protein Adsorption Correlated with Surface Properties of Copolymer Libraries Synthesised as Microarrays*, **M. Taylor**, A.J. Urquhart,* University of Nottingham, UK*, D.G. Anderson, R. Langer,* Massachusetts Institute of Technology*, M.R. Alexander, M.C. Davies*, University of Nottingham, UK

Combinatorial methods have become increasingly popular as a means of material development, allowing rapid discovery and optimisation of new materials. Micro patterned combinatorial material libraries have been shown to be a useful method of screening materials for a number of biological applications. Protein adsorption to surfaces underpins biological response and is therefore of great importance in both implantation and tissue culture situations. Adsorbed proteins effectively translate the structure of a surface into a biological language that ultimately influences the way cells adhere and function. Hence, understanding why and how different proteins adsorb to different surfaces and the effect this has on cell adhesion and growth is of major importance. In this abstract, we report on the adsorption of fluorescently labelled fibronectin to a spatially patterned micro-arrayed library of 480 novel copolymers designed to illicit a range of surface phenomena. Using partial least squares models, protein adsorption has been related to the data generated from the high-throughput surface analysis of the array, including surface chemistry (ToF-SIMS and XPS) and wettability (contact angle, surface energetics), as well as the data derived from the screening of the adhesion and proliferation of the chicken embryonic stem cells to the copolymer library. Interesting correlations between surface phenomena and biological response have been derived from the large data sets, information that will provide important pointers for controlling cellular interactions with such polymeric surfaces.

BI-TuP15 On the Thermodynamics of Protein Adsorption Processes*, **J.C. Hower**, Y. He, S. Jiang*, University of Washington

While significant advances in biocompatible and environmentally benign materials have been made, one of the remaining challenges is to understand surface resistance to protein adsorption. Significant experimental efforts have produced only a small number of nonfouling materials and coatings. Moreover the mechanisms of protein resistance are poorly understood and a majority of new material breakthroughs are made fortuitously. Molecular simulations can aide in material development. By simulating in-silico, one can perform costly experimentation after candidates are selected by initial screening. Molecular simulations also provide access to interactions at the protein-surface-solution interface. We have performed extensive work quantifying the repulsive forces that nonfouling surfaces generate on proteins and analyzing the cause of these forces. Yet, the thermodynamic criterion of adsorption or resistance is the change in free energy as a protein approaches a surface. In this work, molecular simulations were used to calculate the free energy change as model peptides in solution approach surfaces of varying nonfouling ability and to develop simulation-based evaluation criteria. The simulations were supported by protein adsorption experiments. By combining simulations and experiments we verified our simulations and evaluated the relative influence of the surface and hydrating water on the process. This combined approach provides feedback on our simulation parameters and a deeper understanding of the mechanisms of protein resistance and adsorption. Our research has demonstrated a strong link between surface hydration and non-fouling ability. Thus simulations and experiments were conducted to evaluate the hydration of functional moieties representing a wide range of nonfouling abilities. The extent of hydration of biologically relevant functional groups, like oligo-ethylene glycol and sugar alcohols, was evaluated by calculating the partial molar volume change due to hydration. This data was then compared to protein adsorption to self-assembled monolayer surfaces presenting the same functional groups. By using a simple measurement of hydration, it is possible to rapidly screen candidate non-fouling moieties. By combining molecular simulations and experimental techniques, we are able to develop a fundamental description of the interactions present at the molecular and macro scale. This in turn supports rational material design based on desired molecular function.

BI-TuP16 Correlation of Changes in Protein Bioactivity Post-Adsorption to Adsorbed Orientation and Conformation*, **K.P. Fears**, R.A. Latour*, Clemson University

It has long been known that proteins rapidly and irreversibly adsorb onto biomaterial surfaces upon contact with bodily fluids. The structure and bioactivity of the adsorbed protein layer are recognized to be critical factors that influence subsequent cellular responses; however, the molecular mechanisms involved are mostly unknown. The bioactivity of an adsorbed protein could be inhibited due to adsorption-induced conformational changes, orientation effects causing the active site to be sterically blocked, or a combination of both. We have developed experimental methods to measure the bioactivity of an adsorbed protein layer and probe protein orientation and/or adsorption-induced conformational changes. Alkanethiol self-assembled monolayers (SAMs), with different surface chemistries, were used as model surfaces and standard spectrophotometric bioactivity assays were used to measure the percent of protein bioactivity retained post-adsorption. The secondary structure of the adsorbed protein layers was determined using circular dichroism (CD) and compared to the native structure of the proteins. Solvent accessible tryptophan residues were successfully modified using 2-hydroxy-5-nitrobenzyl bromide (Koshland's Reagent) and quantified via spectrophotoscopy. The specific locations of the modified residues are being determined by mass spectrometry to further assess the adsorbed orientation and tertiary structure of the proteins for correlation with changes in their bioactivity.

BI-TuP17 Screening Protein-Surface Interactions with Surface Gradients and TIRF*, **Y.-X. Ding**, B. Wright, V. Hlady*, University of Utah

Macroscopic surface gradients with negative to neutral surface charges were created by reacting 3-mercaptopropyltrimethoxysilane (MTS) with fused silica and selectively oxidizing surface-bound MTS by controlled UV

exposure. XPS analysis and contact angle titrations of the MTS gradients showed that UV oxidation of the MTS sulfhydryl group converts it into a charged sulfonate-like moiety. The MTS gradients were also characterized by AFM in topography, adhesion, and friction modes. The reactivity of unoxidized sulfhydryl groups in the gradient region was assessed by quantitative fluorescence microscopy. The MTS gradients were then used to screen protein interactions with model surfaces. Adsorption kinetics of three human blood proteins, albumin, IgG and fibrinogen, were measured at 1% of their plasma concentrations using a dual channel total internal reflection fluorescence (TIRF) technique. For each protein, two binding experiments were carried out using the same surface gradient sample. In one TIRF channel the solitary binding of one of the fluorescently labeled plasma proteins was observed, and in the other the adsorption of the same was observed from a mixture with the other two unlabeled proteins. The TIRF experiments were later quantified using autoradiography. The adsorption behavior of the three proteins along the sulfhydryl-sulfonate surface gradient was analyzed by taking into account the convective/diffusive transport processes. The on- and off-binding rate constants were obtained as a function of the gradient position by fitting the experimental data to a simple model. Because the unoxidized MTS sulfhydryl can be further derivatized, these techniques have potential to be used as screening tools to study protein-surface interactions on a wide variety of gradient surface chemistries.

BI-TuP18 Detection of Lp-PLA2 as a Biomarker for Atherosclerosis Using Superquenching, ***S. Chemburu***, *Y. Wu,* University of New Mexico, *K. Ogawa, K. Schanze,* University of Florida, *D. Whitten, G.P. Lopez*, University of New Mexico

Lipoprotein associated phospholipase A2 (Lp-PLA2) is being recognized as a new biomarker for the prognosis and diagnosis of atherosclerotic patients. Lp-PLA2 cleaves the sn-2 acyl bond of glycerol-phospholipids yielding a fatty acid and a lysophospholipid as byproducts, which play an important role in the generation of pro-inflammatory moieties. The assays that have been developed for quantifying its catalytic activity or its concentration are time consuming and involve tedious experimental procedures. We have developed a simple bead based fluorescent assay for the quantification of the catalytic activity of Lp-PLA2. Using the layer-by-layer coating of surfaces approach, borosilicate glass beads (5uM dia) were coated with a cationic fluorescent conjugated polyelectrolyte poly(phenylene ethynylene) (PPE). The polymer-coated beads were then covered by a layer of an anionic lipid bilayer that is a natural substrate for PLA2. The lipid bilayer acted as a barrier protecting the fluorescence of PPE from being quenched by anthraquinone disulfonate (AQS). Upon the addition of PLA2, the hydrolysis of the lipid bilayer is catalyzed exposing the PPE to AQS and hence the fluorescence of PPE is turned off. The decrease in fluorescence quenching of the PPE in the presence of the lipid bilayer by AQS has been termed as frustrated superquenching and the authors have used this to develop a simple assay for the quantification of Lp-PLA2 activity.

BI-TuP19 Nanoscale Patterning of Photosynthetic Light Harvesting Proteins, ***N. Reynolds***, *S. Janusz, J. Olsen, C.N. Hunter, G.J. Leggett*, The Univ. Of Sheffield, UK

Here we present the patterning of light harvesting 2 (LH2) complexes from the photosynthetic bacterium Rhodobacter sphaeroides. LH2 complexes consist of circular arrays of bacteriochlorophyll and carotenoid molecules, held together by a cylindrical assembly of polypeptides. As many as 100 LH2 complexes are organised in the membrane to form an interconnected energy transfer networks comprising thousands of bacteriochlorophyll molecules that absorb photons, channelling the excitation energy down an energy gradient towards the reaction centre (RC), leading to a charge separation that drives subsequent biosynthetic reactions in the cell. When removed from the photosynthetic membrane, LH2 complexes retain the ability to absorb light, and they emit the energy as fluorescence. This property has been utilised in order to gain insight into the biological functionality of the LH2 after immobilization. Alkanethiol SAMs on gold surfaces have been used in conjunction with photolithographic techniques to produce patterned assemblies of LH2. Selective exposure of alkanethiols to UV light (wavelength 244 nm) leads to their photo-oxidation to alkylsulfonates, which may be displaced by a second thiol in a solution-phase process. The adsorption of LH2 onto SAMs with a variety of functional groups has been measured in order to determine which surfaces resist non-specific adsorption. In contrast to plasma proteins, which adsorb strongly to most surfaces, simple patterns consisting of hydrophilic and hydrophobic regions may be used effectively to pattern LH2. Covalent attachment to carboxylic acid groups using carbodiimide activation methods is an effective means of immobilising LH2 at the surface. Fluorescence spectroscopy measurements of proteins immobilized by attachment to patterned SAMs have confirmed that biological function is retained, leading to the observation of absorption spectra qualitatively identical to those of complexes in solution. Nanoscale chemical patterns have been fabricated using scanning near-field photolithography (SNP), in which a scanning near-field optical microscope coupled to a UV laser is used to selectively expose regions of a SAM. Using SNP, lines of carboxylic acid functionalised thiols as small as 70 nm have been fabricated in monolayers of perfluorinated thiols, and used to form LH2 structures with a width of less than 100 nm.

BI-TuP20 Molecular Simulation Studies of Protein Interactions with Phosphorylcholine Self-Assembled Monolayers, ***Y. He***, *J.C. Hower, S. Chen, M.T. Bernards, S. Jiang*, University of Washington

We performed molecular simulations to study the interactions between a protein (lysozyme) and phosphorylcholine(PC) self-assembled monolayers (SAMs) in the presence of explicit water molecules and ions. The all-atom simulations were performed to calculate the force generated on the protein as a function of its distance above the SAM surfaces. The structural and dynamic properties of water around PC-SAM surfaces were analyzed. These properties were also compared with those for the oligo(ethylene glycol) (OEG) SAM systems. Results show that the water molecules above the PC-SAM surfaces create a strong repulsive force on the protein as it approaches the surfaces. Further studies show that the dynamics of the water molecules are significantly slowed around both the PC-SAM and OEG-SAM surfaces as compared with that of bulk water, suggesting that the PC-SAM surface generates a tightly bound, structured water layer around their head groups, similar to the OEG-SAM surface. Our results also show that the PC-SAM surface is holding water more strongly than the OEG-SAM surface. The water molecules in the hydration layer of the PC-SAM surface stay longer and reorients slower than those in the hydration layer of the OEG-SAM surface. The most significant difference observed between these two surfaces is that the dipole of non-hydrogen bonded interfacial water molecules reorients much slower on PC-SAM than on OEG-SAM, which can be due to the zwitterionic nature of PC head groups. The application of PC-SAM in bio-lubrication studies will also be discussed.

BI-TuP21 First Direct Observation of Membrane Lipid Asymmetry Induced by Polypeptide Association, ***N. Biswas***, *J.C. Conboy*, University of Utah

Cellular membranes of eukaryotic cells are characterized by a heterogeneous distribution of phospholipids, which is key to many physiological functions. While some membranes (such as the endoplasmic reticulum) are symmetric, others (as the plasma membrane) are asymmetric with phosphatidylethanolamine and phosphatidylserines being primarily localized in the inner leaflet. The loss of asymmetry in plasma membranes has been thought to have direct relevance to numerous physiological and pathological events, such as phagocytosis and cell apoptosis. Although much is know about the biosynthesis of phospholipids, little is known about their mechanism of translocation and even less is known about the mechanism which produces or maintains lipid asymmetry in membranes. The present study shows for the first time, spontaneous development of asymmetry in a DSPC-d_{70}/DSPS (1,2-distearoyl-sn-glycero-3-phosphocholine /1,2-distearoyl-sn-glycero-3-[phospho-L-serine] planar supported lipid bilayer, in the presence of a positively charged polypeptide (polylsyine). The negatively charged DSPS molecules selectively localize in the top leaflet of the bilayer due to the electrostatic association with the polypeptide. The highly surface specific, second order nonlinear spectroscopy, sum frequency vibrational spectroscopy (SFVS), has been used since it offered the opportunity to study the kinetics of unlabeled lipids that were not modified by a fluorescent or spin-probe.

BI-TuP22 Smart Polymers and Cancer Cell Culture: Investigating The Effects of Film Deposition and Cell Culture Parameters on Cellular Behavior, ***J.A. Reed***, *E. Romero, A. Wandinger-Ness, H.E. Canavan*, University of New Mexico

Cell/surface interactions are mediated via the extracellular matrix (ECM). Many important proteins extend into the ECM, such as epidermal growth factor receptor (EGFR). EGFR is often up-regulated in cancer cells, resulting in uncontrollable cell growth and metastasis, which makes them key candidates for in vitro cancer therapeutic models. However, the methods traditionally used to detach cells from surfaces destroy the integrity of proteins in the ECM, resulting in damaged protein and receptors, making them incompatible with the solution-based analysis. In this work, we describe the use of a thermo-responsive polymer, poly(N-isopropyl acrylamide) or pNIPAM, for non-destructive release of cells into suspension. Many cell types, ranging from fibroblasts to epithelial cells, have demonstrated cell sheet detachment on pNIPAM surfaces. However, there is presently very little data on primary cells, cell culture parameters, or the method of film fabrication. To optimize the pNIPAM substrates, pNIPAM films were prepared using different methods (e.g., dissolution in silica vs. plasma deposited films) and compositions (10-40 wt% pNIPAM). The surface chemistry of the resulting films was characterized using X-ray

photoelectron spectroscopy (XPS), film thickness was ascertained via interferometry and XPS, and the thermo-responsivity was determined by contact angle analysis. The cell releasing properties of the films was characterized by incubating baby hamster kidney (BHK) cells to confluency, introducing a variety of solvents (e.g., PBS vs. serum free media) below the LCST, and observing the rate of detachment via microscopy. We find that plasma deposited films exhibited the best detachment behavior when rinsed with PBS and allowed to detach in 4C serum free media.

BI-TuP23 Development of Antimicrobial Materials Based on Surface-Active Biocides in a Resin Matrix, ***R.A. Brizzolara***, NSWC, Carderock Division, *J.H. Wynne*, Naval Research Laboratory, *J. Jones-Meehan*, Department of Homeland Security

The goal of this work is to develop a surface that demonstrates biocidal activity with less than 0.5% biocide concentration in the bulk material. The incorporation of an amphiphilic biocide as a surface-active component in a resin matrix is being investigated as a means of achieving a higher biocide concentration at the surface (and therefore better biocidal efficacy), while maintaining low bulk biocide concentrations. The amphiphilic quaternary ammonium compounds containing both aliphatic and oxyethylene moieties afforded greatest biocidal activity. These molecules were synthesized via simple condensation of a tertiary dimethyl-N-alkylamine with the corresponding halo-oxyethylene. Purity was confirmed using 1H- and 13C-NMR. The biocide-containing urethane coating was dried on glass slides for microbiological analysis and on gold surfaces for x-ray photoelectron spectroscopy (XPS) analysis. Microbiological analysis was performed to determine biocidal efficacy of coating formulations against S. aureus (Gram-positive bacterium). 10 µL of bacterial culture was placed on the surface of the slide. After 2 hours, the slide surface was swabbed. Swab contents were resuspended in appropriate neutralization media, serial dilution was performed with plating on LB agar plates. XPS was used to determine the quantity of biocide at the coating surface compared to the bulk to determine the segregation of biocide to surface, and to correlate surface concentration of biocide to the coating's biocidal efficacy. The N1s binding energy shift between nitrogen in quaternary ammonium and nitrogen in polyurethane was used to differentiate biocide from polyurethane. The XPS analysis demonstrated surface enrichment of two quaternary ammonium-based biocides in a polyurethane coating by approximately a factor of 10 compared to the bulk. The quaternary ammonium-containing materials exhibited increased killing of - S. aureus cells compared to the control (polyurethane containing no biocide). These results indicate that the use of surface-active biocides can result in significant biocidal efficacy with small bulk biocide concentrations. This work was sponsored by the DARPA Defense Sciences Office.

BI-TuP24 Immobilization of Live Salmonella on Abiotic Surfaces for AFM Investigation, ***Z.Y. Suo***, *R. Avci, L. Kellerman, X.H. Yang, D.W. Pascual*, Montana State University

High-resolution AFM images of gram-negative pathogenic Salmonella typhimurium reveal the morphological features of bacterial cells, including CFA/I fimbriae with a diameter of ~3 nm, flagella with a diameter of ~11 nm, and the extracellular polymeric substance surrounding the bacteria. The fine details of the CFA/I fimbriae and the lipopolysaccharides decorating them are clearly resolved when imaged with ultrasharp tips in tapping mode. For studies in liquid, however, it is necessary to immobilize bacterial cells through some sort of "leash," or cross-linker. Live S. typhimurium and their adhesins were successfully immobilized through interactions between bacterial surface antigens and their corresponding antibodies covalently linked to a substrate. Cells immobilized in this way remain viable for hours in PBS buffer and are capable of regenerating if incubated in a growth medium. Immobilized live S. typhimurium cells were imaged in PBS buffer in contact mode and force-volume mode. This approach opens up new fields of investigation, such as quantification of adhesin-receptor interactions, affinity mapping and patterning of bacterial cells on surfaces, which will be discussed in our presentation.

BI-TuP25 Development of Multi-Phasic Scaffolds for Ligament Tissue Engineering via Melt Electrospun Polyurethanes: Cytotoxicity of Melt Electrospun Aliphatic Polyurethane Fibers, ***A. Karchin***, *J.E. Sanders*, University of Washington

Towards the goal of developing electrospun polyurethane (PU) scaffolds for tissue engineering applications, the cytotoxicity of basic aliphatic PUs based on (CH2)4-content diisocyanates, polycaprolactone and 1,4-butane diamine or 1,4-butanediol were tested. These biodegradable polymers were chosen due to their general biocompatibility, excellent mechanical properties, and designed so that in vivo degradation products can be cleared through normal metabolic pathway. Electrospinning from melt, compared to from solution, is an attractive manufacturing process as it allows for the formation of small diameter fibers while eliminating the use of solvents which can be cytotoxic. A two-tiered experimental design was employed to determine the suitability of the specific PUs for use as tissue engineering scaffolds from a biocompatibility perspective. First, the effect of atmosphere, temperature and time at elevated temperature on the polymer cytotoxicity was assessed. Second, an investigation into the relationship between melt electrospinning and cytotoxicity was explored by performing cytotoxicity tests on electrospun meshes. These experiments are useful as a guide for subsequent development of the novel electrospun biohybrid enzymatically biodegradable PUs into a tissue engineering scaffolds.

BI-TuP26 "Smart" Biopolymer for Reversible Stimuli-Responsive Platform of Cell-Based Biochips, ***K. Na***, *O. Kim, J. Jung, J. Lee,* Seoul National University, Korea, *J.W. Park, T.G. Lee,* Korea Research Institute of Standards and Science, *J. Hyun*, Seoul National University, Korea

In the presentation, we describe the genetical synthesis of lysine tagged ELP (ELP-K) with inverse phase transition temperature (Tt) of 30? for fabricating a thermo-responsive culture surface. For the micropatterning of ELP-K a removable polymer template was microcontact-printed on the glass surface derivatized with epoxide. After conjugating ELP-K onto the glass surface followed by dissolving a polymer template, highly resolved ELP-K micropatterns were efficiently created on the surface. The successful micropatterning of the polypeptide was confirmed using ELP-K conjugated with fluorescence dye by confocal microscopy as well as atomic force microscopic images. TOF-SIMS images of ELP-K micropatterns verified the highly resolved ELP microstructure on the surface.

BI-TuP27 Growth Behavior of Fibroblast Cell in Culture Medium Containing Nanoparticles, ***S. Fujita***, *T. Ishizaki, N. Saito, O. Takai*, Nagoya University, Japan

1. Introduction Nanoparticles of metal alloy, metallic oxides, semiconductors and ceramics have unique properties compared with bulk materials since they have high reactivity and catalysis by the large specific surface area. Thus, nanoparticles have been paid attention in the various fields and utilized as electric devices, biomedical materials and cosmetics. On the other hand, it has been reported that nanoparticles could have a harmful effect on human body. For example, nanoparticles such as soot in exhaust fumes are inhaled into lung and cause pulmonary and cardiac diseases. In some recent researches, it is reported that nanoparticles could activate an adverse reaction in the body because they pass through cell walls and move through the blood or lymph vessel. However, nobody knows their interactions with the body in detail. In this study, we aimed to investigate the influence of the nanoparticles on the cell growth and assess the risk of nanoparticles. 2. Experimental procedure Au, Pt, and Ag nanoparticles were synthesized by a wet reduction process. Citric acid was used as a reducing agent. Mouse fibroblast cells (NIH-3T3) were cultured in medium (DMEM, pH: 7) containing the nanoparticles in humidified atmosphere containing 5.0% CO_2 at 37°C for 3 days. The cultured cells were counted using blood cell counting chamber and observed with phase-contrast microscope and transmission electron microscope (TEM). 3. Result Only Ag nanoparticles prevented the cells from growing onto the culture dish. Number of cells after the culture for 72 hours decreased with the increase of concentration of Ag nanoparticles. TEM images showed that the Ag nanoparticles were engulfed into a cell tissue. In addition, the ingested Ag nanoparticles were aggregated around the nuclear. However, such aggregation is not the reason that the cell cannot be cultured in the medium containing Ag nanoparticles, because Au and Pt nanoparticles were also aggregated around the nuclear. At least, Ag nanoparticles are toxic to cells. We believe that nanoparticles may provide us many types of risk; damage of DNA, disappear of proteins and so on. We must investigate the mid- and long-term influence of the nanoparticles on human being.

Marine Biofouling Topical Conference

Room: 4C - Session MB+BI-TuP

Marine Biofouling Poster Session

MB+BI-TuP1 Contact Angle Analysis for Barnacle Adhesives, ***E.R. Holm***, *R.A. Brizzolara*, Naval Surface Warfare Center, Carderock Division

The key step in the accumulation of biofouling on immersed surfaces is the permanent attachment of fouling organisms. Patterns of attachment and adhesion of biofouling, in response to surface properties, vary both among and within species. This variation may be mediated by interactions between surfaces and biological adhesives. We have been studying this interaction for the barnacle, Balanus amphitrite. Our initial approach addresses the propensity of barnacle adhesive to wet modified glass surfaces and commercially-available and experimental silicone fouling-release coatings.

Glass surfaces were modified with various organosilane coatings to produce a range of water wettabilities. We verified attachment of organosilane to glass with XPS. We have been quantifying wetting by barnacle adhesive as contact angle. Preliminary results, for modified glass surfaces, indicated that for hydrophilic surfaces, contact angles for adhesive were equivalent to that for water. As surfaces became more hydrophobic, however, contact angles for barnacle adhesive became more variable than those for water. Individual measurements were occasionally substantially lower than typical water contact angles. Differences in contact angle among adhesive samples may be related to the protein content of the adhesive, which varies strongly among individual barnacles. Results will be related to attachment of larval barnacles and adhesion of adult barnacles. Funded by the NSWC Carderock Division In-House Laboratory Independent Research Program.

MB+BI-TuP2 Poly(ethylene glycol)-based Anti-biofouling Surfaces*, T. Ekblad, G. Bergström, C.-X. Du, T. Ederth, B. Liedberg*, Linköping University, Sweden

This work describes the fabrication, characterization and biological evaluation of homogeneous and patterned hydrogel films, used as model coatings in anti-fouling experiments. The work is a part of an EC-initiative on Advanced Nanostructured Surfaces for the Control of Biofouling, AMBIO. The hydrogels consist of poly(ethylene glycol)-containing methacrylate monomers that are UV-grafted onto solid supports, e.g. silanized glass. The physical and chemical properties of these films have been studied using ellipsometry, FT-IR, AFM and a range of other surface characterization techniques. A key property of the hydrogels is that they appear to be resistant to protein adsorption from complex biofluids, including plasma and serum.[1] These observations encouraged us to test the hydrogels as anti-fouling surfaces. Hydrogels, ca. 30 nm thick, were prepared and evaluated in settlement and removal assays using a range of organisms, including barnacle cyprids of the species Balanus amphitrite, Ulva linza zoospores, Navicula diatoms and the three bacteria species Cobetia marina, Marinobacter hydrocarbonoclasticus and Pseudomonas fluorescens. It is clear from the results that the hydrogel surfaces display excellent antifouling properties. All tested organisms displayed significantly reduced settlement compared to reference coatings. The removal of settled organisms generally appeared to be less affected by the surface coating. Though the relationship is not yet confirmed, this study demonstrates that a surface with low protein adsorption also can have advantageous anti-biofouling properties. The broad-spectrum effect of the hydrogel coating does undoubtedly imply that the selected poly(ethylene glycol) chemistry acts on a fundamental stage in the settlement process of biologically diverse organisms. This stage may be the adsorption of biomolecules from glues released by the settling organisms.

[1] A. Larsson, T. Ekblad, O. Andersson, B. Liedberg, Biomacromolecules 2007, 8, 287-295.

MB+BI-TuP3 The Dynamics of Two Species of Megabalanus (Crustacea: Cirripedia: Balanidae) by a Cellular Automata Model*, M. Apolinário,* PETROBRAS, Brazil*, A. Racco*, LNCC, Brazil

The Cellular Automata (CA) model was used in a computational simulation between an introduced species in Brazilian, Rio de Janeiro State's waters Megabalanus coccopoma and a cryptogenic species Megabalanus tintinnabulum (Crustacea: Cirripedia: Balanidae), obtaining a time series where M. tintinnabulum firstly occurs alone and then it interacts with the entrance of M. coccopoma in the system. The simulation also gets data about spatial distribution of both species and column formation, representing the specimens' assessment one above the other, as it occurs at the natural environment. The results show that total recruitment of each species within the maximum height of the columns is important for the predominance of the introduced species in comparison with the cryptogenic one. The comparison between CA model and natural distribution of both species shows that CA represents significantly the interaction between both species of barnacles in the studied area.

Biomaterial Interfaces

Room: 609 - Session BI-WeM

Nano-Engineered Biointerfaces

Moderator: A. Chilkoti, Duke University

8:00am **BI-WeM1 Multifunctional Quantum Dots for Biomedical Detection and Imaging**, ***X. Gao***, University of Washington **INVITED**

Metal and semiconductor nanoparticles in the 1-50 nm size range are of considerable current interest, not only because of their unique size-dependent properties but also their dimensional similarities with biological macromolecules (e.g., nucleic acids and proteins). These similarities could allow an integration of nanotechnology and biology, leading to major advances in medical diagnostics, prognostics, and targeted therapeutics. In this talk, I present recent development of multifunctional nanostructures for biomedical applications, such as bioconjugated nanoparticles for in vitro ultrasensitive detections and in vivo molecular imaging.

8:40am **BI-WeM3 Lateral Bilayer Fusion to Nanofunctional Probes**, *B.D. Almquist, **N.A. Melosh***, Stanford University

The cell membrane is one of the most vital components of a cell, and crossing through this barrier is a crucial component of biotechnology. Integration of inorganic structures with the cell membrane is poorly understood, and current techniques involve creating holes in or puncturing cell membranes to control access into the cell. However, functionalized materials with nanoscale hybrophobic layers may be able to directly fuse the lipid membrane edge to an inorganic structure, enabling non-disruptive electrical and chemical access into the cell. We have tested whether nanoscale inorganic probes integrate into the hydrophobic core of a lipid bilayer using an AFM probe with hydrophobically functionalized bands 5-20 nm thick at the end of the tip. We quantitatively measure the adhesion strength between the probe and the lipid bilayer, and correlate this molecule-membrane force with the molecular structure. We find the thickness of the nanoscale band and the identity of the hydrophobic molecules alters the ability to fuse to the membrane.

9:00am **BI-WeM4 GaN Nanowires for DNA-Sensing Applications**, *C.P. Chen, A. Ganguly, C.H. Wang, **L.-C. Chen***, National Taiwan University, *C.W. Hsu, Y.-K. Hsu, K.-H. Chen*, Academia Sinica, Taiwan

A novel DNA-sensing system based on GaN nanowires (NWs) is presented coupled with their electrochemical impedance and photoluminescence measurements. GaN is well established now for a variety of optoelectronic applications. However, while its inherent bio-compatibility has also been recognized, its application as bio-sensors has been surprisingly lacking till date. Meanwhile, one-dimensional nanostructures have attracted huge interest as potential building blocks for the future nanoelectronic devices. In this report, GaN NWs are used as a transducer for DNA-sensors, by immobilizing single-strand DNA (ssDNA) molecules through covalent binding using organosilane linker (MPTS). The MPTS-modified GaN NWs exhibited an electrochemical window remarkably wider than those of boron-doped diamond or carbon nanotubes reported to date. The immobilization of ssDNA and the subsequent hybridization to double-strand DNA (dsDNA) were confirmed using confocal microscope. Electrochemical impedance measurement showed that interfacial electron-transfer resistance (Ret), from solution to transducer surface, increased significantly when pristine GaN NWs were immobilized with ssDNA, along with a formation of additional semicircle region at lower frequency in Nyquist plot. The unique appearance of double-semicircle region for ssDNA-immobilized NWs, compared to single-semicircle region for pristine GaN NWs, leads to the idea of formation of double-capacitance layer in series. The phenomenon is more prominent by the appearance of double peaks in the plot of phase angle vs. frequency (Bode plot), the second peak, formed after ssDNA-immobilization, showed further increase under the hybridization to dsDNA, and consequently reduces the overall impedance. Moreover, quenching behavior in photoluminescence of the GaN NWs was distinguishable for the ones immobilized with ssDNA and the same hybridized to dsDNA. Both the technique implies the ability of oligonucleotides, immobilized on the NW-surface, to interact with other biomolecules. The dual and label-free sensing capability in impedance and photoluminescence of GaN NWs makes them effective DNA transducers.

9:20am **BI-WeM5 Functional Protein Chip Nano-Templates by Chemical Lithography and Multivalent Chelator Techniques**, ***A. Turchanin***, *M. El-Desawy, M. Schnietz, A. Gölzhäuser,* University of Bielefeld, Germany, *A. Tinazli, H. Großmann, R. Tampé,* Johann Wolfgang Goethe-University, Germany, *H.H. Solak*, Paul Scherrer Institut, Switzerland

To study protein function and interaction, there is a high demand for specific, stable, highly parallel, and functional protein arrays on solid substrates. To implement this, we propose a protein chip approach based on the combination of electron induced chemical lithography[1] with aromatic self-assembled monolayers (SAMs) and multivalent chelators[2] for high-affinity capturing of His-tagged proteins. Templates for functional and switchable His-tagged protein architectures were created by electron induced changes in 4'-nitro-1,1'-biphenyl-4-thiol SAMs on gold (NBPT). Chemical nanopatterns were generated in SAMs by both electron beam lithography (EBL) and extreme UV interference lithography (EUV-IL). As a model system carboxy derivative tris-NTA chelators were studied. Different steps of the protein chip assembly (fabrication of amino terminated cross-linked areas, generation of protein repellent EG3-OH thiols matrix, grafting of tris-NTA chelators, and chelating efficiency of Ni(II) ions) were characterized in detail by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). The functionality of the generated protein chips was shown in situ, under physiological conditions by AFM and scanning fluorescence microscopy measurements via specific, homogeneous, oriented and reversible immobilization of His_6-tagged 20S proteasome and fluorescence labelled His_{10}-tagged maltose binding proteins (MBP). We will present highly parallel large area (~10 mm^2) protein arrays with the lateral dimensions of periodic features ranging from 1000 nm to 50 nm.

[1]A. Gölzhäuser, W. Eck, W. Geyer, V. Stadler, T.Weimann, P. Hinze, M. Grunze, Adv. Mat. 13 (2001) 806

[2]A. Tinazli, J. Tang, R.Valiokas, S. Picuric, S. Lata, J. Piehler, B. Liedberg, R. Tampé, Chemistry 11 (2005) 5249.

9:40am **BI-WeM6 Protein Patterning by Scanning Near-Field Photolithography**, ***G.J. Leggett***, *R.E. Ducker, M. Montague, K.S.L. Chong*, University of Sheffield, UK

Photolithography is a convenient and rapid route to the fabrication of patterned self-assembled monolayers for the control of biological organisation. Alkanethiols may be photo-oxidised by exposure to light with a wavelength of 244 nm to yield soluble sulfonates which may be displaced by complementary thiols to yield clean, well-defined chemical patterns. Protein patterning is complicated by the problems of non-specific adsorption. Most proteins adhere to most surfaces, rendering protein patterning difficult. Oligo(ethylene glycol) (OEG) terminated self-assembled monolayers are attractive because they resist protein adsorption very effectively. Here we have explored the possibility of patterning OEG-terminated SAMs using photolithography. Rates of photo-oxidation of OEG-terminated thiols have been measured using contact angle goniometry, static SIMS and friction force microscopy. The kinetics observed appear to be different from those observed for other thiols. The mechanism appears to be complex. Over longer exposures, photo-oxidation of the head group occurs. Subsequent patterning through a mask yields well-defined structures that consist of either methyl or carboxylic acid terminated thiols in regions exposed to UV light, to which proteins may, respectively, be adsorbed or covalently bound, and protein-resistant OEG-terminated adsorbates over the rest of the surface. Exposure using a near-field scanning optical microscope (scanning near-field photolithography, SNP) yields nanometre scale structures. At short exposures, photodegradation of the OEG chain occurs leading to the formation of aldehyde groups that covalently bind proteins with high efficiency. This provides a very convenient single-step route to the introduction of a reactive functional group, in a spatially selective fashion, to a protein resistant OEG monolayer. The amount of streptavidin bound to such a photo-modified monolayer is nearly as great as that bound using well-established carbodiimide-based methods on carboxylic acid terminated monolayers. Significantly, the photodegradation of the OEG terminal groups in this process is much more rapid than the oxidation of the thiol head-group and is, moreover, capable of excitation at longer wavelengths, where photo-oxidation of the thiol sulfur atom is no longer a possibility. Photopatterning thus appears to be a simple and versatile route to protein patterning.

10:40am **BI-WeM9 Multicomponent Nanoparticles for Controlled Intracellular Delivery to Targeted Cells**, *J.M. Bergen, I.K. Park, E. Kwon, **S.H. Pun***, University of Washington **INVITED**

Gene therapy offers the possibility of treating diseases by altering the protein expression profiles of affected cells. Nucleic acids can be condensed

by complexation with cationic polymers to nanoparticle structures called "polyplexes". These synthetic vehicles can be used to accomplish gene delivery but are usually limited in their applications due to poor delivery efficiencies in non-dividing cells. I will describe our work on developing multicomponent polyplexes that incorporate biologically-derived peptides that facilitate delivery. Peptides that mediate neuron-specific uptake and endosomal release were incorporated into polyplexes. The intracellular trafficking of these materials was also investigated by live cell imaging in compartmented chambers. Incorporation of functional peptides improves delivery efficiency to cultured, neuron-like PC-12 cells.

11:20am **BI-WeM11 Femtosecond Laser Ablation to Create Nanometer-Scaled Cell Adhesion Ligand Patterns**, ***R.C. Schmidt**,* UC Berkeley/UC San Francisco Joint Graduate Group in Bioengineering*, D.H. Hwang, C.P. Grigoropoulos,* UC Berkeley*, K.E. Healy*, UC Berkeley/UC San Francisco Joint Graduate Group in Bioengineering

The goal of our project is to fabricate interfaces for mammalian cell culture that control cell fate via the spatial distribution of the individual focal adhesions cells use to interrogate the interface. To create nano-scale cell adhesion sites on a surface, a thin protein adsorption resistant polyethylene glycol (PEG) brush layer was synthesized via surface initiated atom transfer radical polymerization (SI-ATRP). The surface chemistry was verified with XPS, showing strong oxygen and carbon peaks consistent with a PEG film, and thickness of the dry film in air was calculated to be 10nm using a quartz crystal microbalance with dissipation (QCMD). The film was selectively ablated using focused femtosecond laser pulses, exposing the underlying quartz substrate as centers for adsorption or grafting of cell-adhesive molecules. Preliminary results at a wavelength of 400nm with a 50X objective demonstrated spatial resolution approaching 200nm based on atomic force microscopy (AFM) scanning of the ablated features. The practical resolution limit can be further improved (~10-100nm) by utilizing higher magnification lenses at shorter wavelengths or processing in the optical near-field. This technique allows us to generate arbitrary nanoscale protein patterns on the benchtop without specialized processing environments. These nanostructured surfaces will eventually allow us to decouple the effects of cell size and shape, focal adhesion placement, and ligand density on cell fate decision by directly controlling the number and area of focal adhesion complexes formed. Each variable can be modulated independently to determine the effects on cellular function and fate determination.

11:40am **BI-WeM12 Neuron Pathfinding on Functionalized Patterned, Gradient, and Fiber Biomaterial Surfaces**, *W.M. Theilacker, M.E. Boggs, S.K. Mbugua, S.P. Sullivan,* Univ. of Delaware*, D.E. Willis,* Nemours Biomedical Res. of Alfred I duPont Hospital for Children*, K.W. Dabney,* Alfred I duPont Hospital for Children*, J.L. Twiss,* Nemours Biomedical Res. of Alfred I duPont Hospital for Children*, **T.P. Beebe, Jr.***, Univ. of Delaware

This paper will present the recent results from a collaborative study that is aimed at developing novel growth-promoting substrates for injured and damaged neurons, with an emphasis on understanding the mechanisms of substrate-neuron interactions and the resulting modulation of intra-axonal signal transduction. Axons regenerating in vivo must traverse from a permissive into a non-permissive environment. We use the permissive environment of novel surface-grafted 2-D and 3-D materials to increase the capacity for axons to traverse into a non-permissive growth environment. We have generated novel patterned and well characterized 2-D and 3-D biomaterial growth substrates that mimic the environment encountered by the regenerating axons in the injured spinal cord. This is accomplished by engineered patterns and gradients with tailored composition of growth-promoting extracellular matrix molecules. We will also describe the results of studies aimed to observe and mimic how permissive growth substrates directly modulate axonal-substrate interactions and intra-axonal signal transduction. The program incorporates elements of surface chemistry, surface analysis, cell culture, optical microscopy, and neuroscience.

12:00pm **BI-WeM13 XPS, ToF-SIMS, NEXAFS and SPR Characterization of Nitrilotriacetic Acid-Terminated Self-Assembled Monolayers For Controllable Immobilization of Proteins**, ***F. Cheng****, L.J. Gamble, D.G. Castner*, University of Washington

For immobilization of proteins onto surfaces in a specific and controlled manner it is important to start with a well-defined surface that contains specific binding sites surrounded by a nonfouling background. For immobilizing histidine-tagged (histagged) proteins, surfaces containing nitrilotriacetic acid (NTA) headgroups and oligo(ethylene glycol) (OEG) moieties are a widely used model system. The surface composition, structure and reactivity of mixed NTA/OEG self-assembled monolayers (SAMs) on Au substrates were characterized in detail using X-ray photoelectron spectroscopy (XPS), near-edge X-ray absorption fine structure spectroscopy (NEXAFS), time-of-flight secondary ion mass spectrometry (ToF-SIMS) and surface plasmon resonance (SPR) biosensing. XPS results for sequentially adsorbed NTA thiols followed by OEG thiols showed that OEG molecules were incorporated into a incompletely formed NTA monolayer until a complete mixed SAM was formed. Surface concentration of NTA headgroups was estimated to be 0.9 molecule / nm^2 from XPS results. Angle-resolved XPS and polarization-dependent NEXAFS results both indicated NTA headgroups were slightly reoriented toward an upright position upon OEG incorporation. Nitrogen-containing and OEG-related secondary ion fragments from the ToF-SIMS experiments confirmed the presence of NTA headgroups and OEG moieties in the monolayer. These fragments along with secondary ion amino acid fragments are being used to investigate the orientation and conformation of histagged proteins immobilized onto the NTA/OEG SAMs. SPR measurements of a histagged, humanized anti-lysozyme variable fragment (HuLys Fv) immobilized onto Ni(II) -treated mixed NTA/OEG SAMs demonstrated the reversible, site-specific immobilization of histagged HuLys Fv (170 ± 7 ng / cm^2) with strong binding affinity (approximately 43 nM). The mixed NTA/OEG SAMs without Ni(II) treatment exhibited good resistance to nonspecific adsorption of histagged HuLys Fv (< 2 ng / cm^2).

Understanding Biointerphases and Magnetism with Neutrons Topical Conference

Room: 618 - Session NT+BI-WeM

Phospholipid Bilayers and Membranes

Moderator: M. Tanaka, University of Heidelberg

8:00am **NT+BI-WeM1 Tethered Bilayer Lipid Membranes in Biomedical Research: Lessons from Neutron Scattering**, ***M. Lösche****, F. Heinrich,* Carnegie Mellon University*, D.J. McGillivray,* The Australian National University*, G. Valincius,* Institute of Biochemistry, Vilnius, Lithuania*, Y. Sokolov, J.E. Hall*, UC Irvine **INVITED**

Tethered bilayer lipid membranes (tBLMs) on solid supports hold potential to mimic biological membranes. Molecular-scale studies of the interactions of peptides and proteins with membranes provide ample opportunities in biophysical and biomedical research. Membrane stabilization by the proximity of a solid substrate provides resilience to the system, but has often at the same time introduced severe problems. A prerequisite, for example, for tBLM characterization by scattering and electrochemical techniques is a low defect density of the membrane. Only then is it possible to quantify minor structural and functional changes induced by, e.g., protein interaction with the membrane. We have optimized a membrane architecture on molecularly flat gold surfaces which meets all these challenges. Different lengths of the hydrophilic poly(ethylene glycol) (PEG) spacer that controls the structure of the inner monolayer leaflet provide highly hydrated sub-membrane spaces between 20 Å and 60 Å in thickness, as determined by neutron reflection. Such tBLMs may be composed of charged or zwitterionic lipids with various chain saturation, and can include cholesterol. The membranes are highly insulating and are routinely probed with electrochemical impedance spectroscopy (EIS). As an example for ongoing biomedical research we will discuss the interaction of soluble prefibrillar β-amyloid oligomers with tBLMs and compare the impact of the peptide on such membranes with that of a pore forming bacterial exotoxin, Staphylococcus aureus α-hemolysin.

8:40am **NT+BI-WeM3 Study of Fluctuation and Destabilization of Single Phospholipidic Bilayer by Neutron and X-ray Scattering**, ***T. Charitat****,* CNRS-Université Louis Pasteur, France*, S. Lecuyer*, Harvard University **INVITED**

Supported bilayer are interesting model systems for biologist and present also fascinating physical properties. We investigate experimentally these dynamical properties on floating bilayer. First, the equilibrium structures of single and double bilayers are studied by neutron reflectivity. The submicronic fluctuation spectrum of a floating bilayer is determined by off-specular X-ray scattering: surface tension, bending modulus and, for the first time with this technique, inter-membrane potential. Using fluorescence microscopy, we show that this single bilayer can be completely destabilized leading to well control vesicles formation. Destabilization can occur either at the main gel-fluid transition of the lipids, and can be interpreted in terms of a drop of bending rigidity, or under an AC low-frequency electric field applied in the fluid phase. In that last case we also study the effect of the electric field at the molecular length scale by neutron reflectivity. In both cases, the destabilization leads to the formation of relatively monodisperse vesicles, which could give a better understanding of the formation mechanism.

9:20am **NT+BI-WeM5 Protein-induced Pores in Membranes Detected and Studied by Neutron Scattering**, ***H.W. Huang***, Rice University **INVITED**

Gene encoded antimicrobial peptides kill bacteria by forming pores in the bacterial membranes. Apoptotic protein Bax forms pores in the outer mitochondrial membrane to release the apoptosis-inducing factor cytochrome c from mitochondria. The evidence of pore formation in membranes is usually ion conduction or leakage. The structure of a pore in a fluid membrane is difficult to detect or measure by conventional methods such as electron microscopy. Neutron scattering is uniquely suited for such structural studies. We will show neutron scattering from membrane pores made by antimicrobial peptides, alamethicin, magainin, protegrin as well as by bee venom toxin melittin. Surprisingly, these peptides form two different kinds of transmembrane pores first detected by neutron methods.

10:40am **NT+BI-WeM9 Using Neutron Spectroscopy to Study Collective Dynamics of Biological and Model Membrane Systems**, ***M.C. Rheinstadter***, University of Missouri-Columbia **INVITED**

The spectrum of fluctuations in biomimetic and biological membranes covers a large range of time and length scales, ranging from the long wavelength undulation and bending modes of the bilayer with typical relaxation times of nanoseconds and lateral length scales of several hundred lipid molecules, down to the short-wavelength, picosecond density fluctuations involving neighboring lipid molecules. New developments and improvements in neutron scattering instruments, sample preparation and environments and, eventually, the more and more powerful neutron sources open up the possibility to study collective excitations, i.e. phonons, in artificial and biological membranes. The goal of this project is to seek relationships between collective dynamics on various length scales on the one hand, and macroscopic phenomena such as trans-membrane transport, pore opening, and membrane fusion on the other hand. The combination of various inelastic neutron scattering techniques enlarges the window of accessible momentum and energy transfers - or better: accessible length and time scales - and allows one to study structure and dynamics on length scales ranging from the nearest-neighbor distances of lipid molecules to length scales of more than 100 nm, covering time scales from about 0.1 ps to almost 1 µs. The fluctuations are quantified by measuring the corresponding dispersion relations, i.e. the wave vector-dependence of the excitation frequencies or relaxation rates. Because biological materials lack an overall crystal structure, in order to fully characterize the fluctuations and to compare experimental results with membrane theories, the measurement must cover a very large range of length and time scales. By using multiple instruments, from spin-echo to triple-axis spectrometers, we have successfully probed these fluctuations over the desired range of length and time scales.[1-5]

[1]M.C. Rheinstädter, C. Ollinger, G. Fragneto, F. Demmel and T. Salditt, Phys. Rev. Lett. 93, 108107, 1-4 (2004).

[2]Maikel C. Rheinstädter, Wolfgang Häußler and Tim Salditt, Phys. Rev. Lett. 97, 048103, 1-4 (2006).

[3]Maikel C. Rheinstädter, Tilo Seydel, Franz Demmel and Tim Salditt, Phys. Rev. E 71, 061908, 1-8 (2005).

[4]Maikel C. Rheinstädter, Tilo Seydel and Tim Salditt, Phys. Rev. E 75, 011907, 1-5 (2007)

[5]Maikel C. Rheinstädter, Tilo Seydel, Wolfgang Häußler and Tim Salditt, J. Vac. Soc. Technol. A 24, 1191-1196 (2006).

11:20am **NT+BI-WeM11 The Coupling between Hydration-Water and Protein Dynamics as Studied by Neutron Scattering**, ***M. Weik***, IBS, CEA-CNRS-UJF, France **INVITED**

The dynamics of proteins is influenced by motions of water molecules at the protein-solvent interphase. However, details about the dynamical coupling remain to be elucidated. Neutron scattering is particularly well-adapted to study macromolecular motions on the ns-ps time scale and their coupling to hydration-water dynamics. Indeed, elastic incoherent neutron scattering is sensitive to hydrogen/deuterium isotope labelling with the scattering cross-section of hydrogen being about 40 times larger than that of deuterium. Consequently, studying a completely deuterated protein hydrated in H_2O gives access to the dynamics of hydration water. Conversely, an identically prepared sample of hydrogenated protein hydrated in D_2O yields information on protein dynamics only, thus enabling a direct comparison between hydration water and protein motions. We studied the coupling between hydration-water and protein dynamics in a biological membrane (purple membrane (PM)) and a soluble, globular protein (maltose binding protein (MBP)) by measuring mean square displacements of hydrogen atoms in the temperature range from 20 to 300 K. Hydration-water in both PM and MBP undergoes a dynamical transition at 200 K, evidenced as a break in atomic mean square displacements as a function of temperature (Wood, Frölich, Plazanet, Kessler, Moulin, Härtlein, Gabel, Oesterhelt, Zaccai & Weik, unpublished results). In the case of PM, this dynamical transition corresponds to the onset of long-range translational diffusion of water molecules as evidenced by neutron diffraction.[1] When atomic mean square displacements of hydration-water molecules become as large as those of protein atoms, a dynamical transition appears at 250 K in PM and at 230 K in MBP. Our results shed new light on the coupling between hydration-water and protein motions and suggest that they are coupled at room temperature, yet decoupled at cryo-temperatures.

[1]Weik, M., Lehnert, U. and Zaccai, G. (2005) Liquid-like water confined in stacks of biological membranes at 200 k and its relation to protein dynamics. Biophys J., 89, 3639-3646.

Applied Surface Science

Room: 610 - Session AS+BI+NS-WeA

Fabrication and Characterization of Functional Soft Material Surfaces

Moderator: R.T. Haasch, University of Illinois at Urbana Champaign

1:40pm **AS+BI+NS-WeA1 UPS Work Function Measurements on Polymers Combined with C60 Depth Profiling**, ***S. Raman***, *J. Moulder, J.S. Hammond,* Physical Electronics*, N. Sanada, M. Suzuki*, ULVAC-PHI, Inc.

The performance of ultra thin organic films in organic LED's (OLED) is dependent on the work functions of the electrodes and polymers forming the OLED junctions. Historically, the work functions have frequently been derived from the secondary electron emission edges measured with UPS. The recent applications of C60 ion beams for the sputter removal of a surface layer of many organic materials, leaving the remaining surface with minimal chemical damage, have offered a new tool for studying surface modified polymers. By combining these two techniques, the work function and composition as a function of depth of polymers targeted for OLED applications can be characterized by XPS and UPS. The surface characterization of OLED component polymers exposed to deleterious environments will also be discussed. The possible chemical surface damage induced by the C60 ion beams will be examined by both XPS and UPS spectra and secondary electron emission edge spectra.

2:00pm **AS+BI+NS-WeA2 Patterning and Bonding of Poly(dimethylsiloxane) A Simple New Method for Creating Optically Transparent Biocompatible Surfaces and Robust Microfluidic Devices**, ***P.R. Norton***, *N. Patrito, J. McLachlan, J. Chan, S. Faria, S. Tadayyon*, University of Western Ontario, Canada

Our group has developed a simple protocol to prepare inexpensive, single-component substrates capable of confining cell attachment and growth. In the presence of an argon plasma, thin metal films are deposited onto poly(dimethylsiloxane) (PDMS). Removal of the metal layer exposes regions of the polymer surface that are enriched in oxygen and promote the adhesion of fibroblast, epithelial and myoblast cells. This method produces bioactive arrays of controlled size (down to scales in the order of µm), shape, pitch and symmetry on which cells can be grown to confluency. The treated material is storable and can be activated just before use; this eliminates stability problems inherent in a number of previously reported PDMS surface treatments, most notably oxygen plasma modification. The patterned arrays offer highly adaptable means to probe cell-cell interactions, cell motility and cell signaling in response to varied spatial or geometric organization and they are being incorporated into microfluidic channels for combined optical and proximal probe studies of live cells. Serendipitously, this surface treatment alters the mechanical properties of PDMS, rendering the modified material sensitive to tensile stresses imposed by cells. Cellular traction forces generate nanoscale ripples in the elastic substrata which extend outward from the cell bodies and which can be imaged by dark-field microscopy and AFM. Detailed analyses of these ripples can potentially provide a direct measure of cellular traction forces and mechanical signaling. In related experiments, we have also developed a novel means of bonding PDMS to a host of materials relevant to microfluidic device fabrication, including glass, Si, SiO2 and polystyrene. To quantify the adhesive strength, closed PDMS-glass and PDMS-PDMS microfluidic devices were fabricated and subjected to tensile and leakage testing. The data indicate a significant improvement in performance over previously reported bonding technologies, resulting in the production of more robust, longer-lasting microfluidic devices and the concomitant possibility of using higher pressures and flow-rates.

2:20pm **AS+BI+NS-WeA3 Interfacial Structure of Polymer Brush and Gel Investigated by Sum Frequency Generation Spectroscopy**, ***K. Uosaki***, *H. Noguchi, S. Nihonyanagi, H. Minowa, R. Yamamoto*, Hokkaido University, Japan

Polymer brush and gel plays very important roles in biological systems. Information on the interfacial structure is essential to understand the function of these materials. Here we employed sum frequency generation (SFG) spectroscopy, which is known to possess high surface specificity, to investigate the molecular orientation/conformation of polymer brush under various environment and water structure at PVA gel/solid interface. SFG spectra of alkylated poly (vinyl pyridine) in contact with dry nitrogen, water vapor, and liquid water were obtained. The peaks due to CH vibration of CH3 dominated in nitrogen, showing that the side chains are highly ordered. When the polymer layer is in contact with water vapor, in addition to the two peaks due to CH3 group, two peaks of CH2 group were observed, indicating that many gauche defects existed. The SFG spectrum of the polymer brush in contact with liquid water showed no peaks in CH stretching region except for small shoulder due to the ring CH modes, indicating the alkyl side chain was completely disordered. SFG spectra of the polymer brush in OH stretching region were also obtained. A broad band assigned to the OH stretching was observed in water vapor. Two bands corresponding to the OH stretching of the interfacial water molecules at 3200 cm-1 due to "ice-like water" and at 3400 cm-1 due to "liquid-like water" dominated in liquid water. Poly vinyl alcohol (PVA) gel is considered to be one of the possible candidates for an artificial articular cartilage in artificial joints because of very low friction between PVA gel and solid. SFG measurement proved that while the fractions of the "ice-like" and "liquid-like" water were almost equal at water/quartz interface, the fraction of the "liquid-like" water became much higher when the PVA gel was pressed against the quartz surface, showing that the weakly hydrogen bonded water dominate at PVA gel/quartz interface where friction is very low. SFG measurement in Ar showed only weakly hydrogen bonded water was present at the PVA gel/quartz interface. The effect of PVA gel contact was less obvious at the octadecyltrichlorosilane (OTS) coated quartz, where the friction is much higher. These results suggest the important role of weakly hydrogen bonded water for very small friction at PVA gel/solid interface. In conclusion, SFG is demonstrated to be a very useful technique to characterize molecular structure at solid/liquid interfaces including biological interfaces.

2:40pm **AS+BI+NS-WeA4 Multiphoton Patterning of Planar and Topographically Complex Surfaces for Control of Photon, Electron, and Chemical Transport**, ***P.V. Braun***, University of Illinois at Urbana-Champaign **INVITED**

Multiphoton patterning enables fabrication of complex structures with minimum feature sizes on the order of the volume of the focal point. Using a pulsed laser source coupled to a laser scanning confocal microscope, we have concurrently written and imaged various complex structures and patterns. Recently, along with formation of complex structures, we have initiated efforts to locally modify the surface chemistry of both planar and topographically complex (three-dimensional) surfaces to create pathways for photon, electron, and chemical transport. A series of organic chemistries have been developed to enable this patterning, which revolve around the grafting of photoswitchable molecules or polymer brushes to the various surfaces. Once the local chemistry of the surface has been modulated, this chemistry can be amplified through quantum dot attachment, electroless plating, or surface initiated polymerization to create two and three-dimensional patterns for control of photon, electron, and chemical transport. Colloidal crystals and porous glasses are used as model three-dimensional substrates; standard materials are used for two-dimensional structures.

4:00pm **AS+BI+NS-WeA8 Chemical Modification of Self-Assembled Monolayer Surfaces using Soft-Landing of Mass-Selected Ions**, ***P. Wang***, *O. Hadjar, J. Laskin*, Pacific Northwest National Laboratory

Stable immobilization of peptides on solid supports plays an important role in biochemistry. Existing techniques for linking peptides to surfaces are based on a variety of solution-phase synthetic strategies and require relatively large quantities of purified material. Here, we report a novel approach for preparation of peptide arrays on self-assembled monolayer (SAM) surfaces using soft-landing (SL) of mass-selected ions. This approach takes advantage of the exceptional selectivity in preparation of projectile ions by mass spectrometry. We demonstrate efficient reactive landing (RL) of several model peptides onto the SAM of N-hydroxysuccinimidyl ester terminated alkylthiol on gold (NHS-SAM). This method introduces unprecedented selectivity and specificity into the surface preparation step by eliminating the effect of solvent and sample impurities on the quality of the film. Peptide ions produced by electrospray ionization are mass-selected and deposited onto SAM surfaces using a novel ion deposition chamber designed and constructed in our laboratory. NHS-SAM, which readily reacts with accessible primary amino groups in proteins or peptides by forming amide bonds, is used as a target for soft-landing experiments. Freshly prepared SAMs and modified surfaces are characterized ex situ using time-of-flight secondary ion mass spectrometry (ToF-SIMS) and infrared reflection absorption spectroscopy (IRRAS). Mass-selected ions of model peptides including cyclo(-RGDfK-), GRGDSPK and RGDGG were soft-landed onto the NHS-SAM surface.

ToF-SIMS and IRRAS characterization suggested efficient covalent binding between the two lysine-containing peptides and the NHS-SAM by the formation of an amide bond through the lysine side chain. Systematic studies were carried out to understand the factors that affect the efficiency of reactive landing. We found that the reaction takes place upon collision and is promoted by the kinetic energy of the ion. The reaction yield is independent of the charge state of the projectile ion suggesting efficient neutralization of peptide ions upon collision. Chemical reactivity and physical properties of the SAM surface are also important factors that affect the outcome of RL. RL of mass- and energy-selected peptide ions on surfaces provides a highly specific approach for covalent immobilization of biological molecules onto SAM surfaces.

4:20pm **AS+BI+NS-WeA9 TOF-SIMS Analysis of Polypropylene Films Modified by Isotopically Labeled Methane Flames**, ***S.J. Pachuta***, *M.A. Strobel*, 3M Company

Flame treatment is a common industrial process for modifying polymer surfaces. Surfaces exposed to flames are known to oxidize, but studies of the oxidation mechanism have been largely confined to correlating simple surface properties with models of the flame composition due to the lack of direct experimental data on the flame-surface interaction. In this work, polypropylene film surfaces were oxidized by exposure to a flame fueled by isotopically-labeled methane (CD_4). The isotopic sensitivity of time-of-flight secondary ion mass spectrometry (TOF-SIMS) was then used to gain new insights into the mechanism of flame treatment. TOF-SIMS analysis indicates that much of the oxidation of polypropylene occurring in fuel-lean flames is not accompanied by deuteration, while for polypropylene treated in fuel-rich flames, deuteration is extensive, and some of the affixed oxygen is deuterated. These observations imply that O_2 is the primary source of affixed surface oxygen in fuel-lean flame treatments, but that OH may be a significant source of affixed oxygen in fuel-rich flame treatments. Application of principal component analysis (PCA) and multivariate curve resolution (MCR) to the TOF-SIMS data was found to provide information beyond that which could be obtained by traditional peak-ratio methodology.

4:40pm **AS+BI+NS-WeA10 The Analysis of Oxidation Profiles in Elastomers Using ToF-SIMS**, ***J.A. Ohlhausen***, *M.C. Celina*, *M.R. Keenan*, Sandia National Laboratories

As elastomeric materials age, their mechanical properties can change such that they do not perform their desired function. It is important to understand the aging behaviour of such elastomers, so that predictive aging models can be developed. In that light, much work has been done to understand oxygen diffusion limited aging processes under accelerated aging conditions for a range of elastomers.[1-2] In these studies, mechanical properties as a function of depth were determined under differing temperature/time conditions. In addition, chemical changes were measured by analyzing microtomed sections using FTIR. Unfortunately, these measurements were difficult to perform on carbon-black filled samples and were also relatively time consuming. Ideally, an alternative method is needed to measure the chemical oxidation profiles of commercial o-rings containing fillers in a quick and efficient manner. In this talk, the usefulness of ToF-SIMS as a technique to measure the position-dependent extent of oxidation in filled elastomers will be discussed. Accelerated aging in 18O is used to demonstrate the ability of SIMS to directly measure the extent of oxidation. Examples of 18O- cross section line scans in aged elastomers will be discussed and compared to known diffusion limited degradation mechanical profiles. It will be shown that filled elastomers can be analysed using these methods. Additionally, the ability to measure oxidation profiles in air-aged samples will also be shown. Thus, ToF-SIMS is seen as a rapid evaluation tool for the measurement of elastomer oxidation for engineered elastomers. The benefits and limitations of the technique will be discussed. Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

[1] M. Celina, J. Wise, D. K. Ottesen, K. T. Gillen, R. L. Clough, Polymer Degradation and Stability 60 (1998) 493-504

[2] M. Celina, J. Wise, D. K. Ottesen, K. T. Gillen, R. L. Clough, Polymer Degradation and Stability 68 (2000) 171-184 .

5:00pm **AS+BI+NS-WeA11 Surface Chemical Analysis of Nano-Scaled r.f. Plasma Polymer and Co-Polymer Films by using a Combination of "In-Situ"and Ex-Situ Characterization Tools: Hydroxylated and Aminated Surfaces by XPS, ToF-SIMS and NEXAFS Spectroscopy**, ***W.E.S. Unger***, *A. Lippitz*, *S. Swaraj*, *E. Yegen*, Federal Institute for Materials Research and Testing (BAM), Germany

The formation of plasma-polymerized materials made from organic molecules is a technologically highly attractive way to obtain films with unique properties. Surface properties like bio-compatibility, wettability, etc., can be adjusted by tailoring the chemical functionalization. A controlled deposition of those films requires the development of surface analytical procedures which are able to derive useful information on relevant parameters. This can be a hard job for an analyst because plasma-polymerized or plasma-modified materials are extremely complex samples. Their complexity is caused by the co-existence of a relatively high number of chemical species. This leads to a kind of mixture analysis at surfaces but without the possibility of a separation step as it is possible, e.g., by using GC-MS techniques in the analysis of organic mixtures. Relevant parameters for film characterization are primarily (1) qualitative and (2) quantitative determination of functional groups. However there are other important parameters as for instance (3) the cross-linking and branching within the films or even the concentrations of (4) radicals and (5) unsaturated species in the films. It is well known that plasma-polymerized materials may undergo ageing processes. It is assumed that many of them will be initiated by radicals in the films. These radicals are inherently produced by plasma processing. Approaches have to be developed enabling a study of ageing processes on the molecular level including the respective reaction kinetics. So-called "in-situ" techniques of surface chemical analysis are required to investigate the real fresh state of samples. Using a selection of hydroxylated or aminated model plasma polymers and co-polymers it will be demonstrated how analytical approaches based on a combination of photoelectron spectroscopy (XPS), secondary ion mass spectrometry (SIMS) and x-ray absorption spectroscopy (XAS) may provide solutions for the analytical challenges summarized above.

Biomaterial Interfaces

Room: 609 - Session BI-WeA

Nucleic Acid Sequencing and Technology

Moderator: L.J. Gamble, University of Washington

1:40pm **BI-WeA1 Surface Initiated Enzymatic Polymerization of DNA**, ***A. Chilkoti***, *D. Chow*, *S. Zauscher*, Duke University

We demonstrate a technique to synthesize DNA homopolymers on a surface using surface-initiated enzymatic polymerization (SIEP) with terminal deoxynucleotidyl transferase (TdTase), an enzyme that repetitively adds mononucleotides to the 3' end of oligonucleotides. The thickness of the synthesized DNA layer was found to depend on the deoxymononucleotide monomer, in the order of dATP > dTTP >> dGTP - dCTP. In addition, the composition and the surface density of oligonucleotide initiators were also important in controlling the extent of DNA polymerization. Poly(dTTP) synthesized by SIEP was capable of binding to antibodies specific to oligomers of dTTP, indicating that the DNA homolayer is fully functional. TdTase-mediated SIEP can also be used to grow spatially defined three-dimensional DNA structures by soft-lithography and by E-beam nanolithography, and is a new tool for bioinspired fabrication at the micro- and nano-scale.

2:00pm **BI-WeA2 Genome Sequencing with Polony Technology**, ***J.S. Edwards***, University of New Mexico **INVITED**

The resounding success of the Human Genome Project (HGP) clearly illustrates how early investments in developing cost-effective methods of biological data acquisition can have tremendous payoffs for the biomedical community. Over the course of a decade, through refinement, parallelization, and automation of established sequencing technologies, the HGP motivated a 100-fold reduction of sequencing costs, from $10 per finished base to $0.10 per finished base. The relevance and utility of sequencing and sequencing centers in the wake of the HGP has been a subject of recent debate, however, I maintain that the completion of the human genome marks the end-of-the-beginning, rather than the beginning-of-the-end, of the era of DNA sequencing and, more generally, the era of nucleic-acid (NA) technologies. For a wide range of biomedical goals, a strong need is evolving for low-cost NA technology, and I will describe our progress in using polony technology to cheaply and rapidly re-sequence a human genome. The list of realized and potential applications for this type of high-throughput sequencing technology is rich and growing.

2:40pm **BI-WeA4 In Situ Study of Ionic Strength and Probe Coverage Influences on DNA Hybridization using Cyclic Voltammetry**, ***P. Gong***, *K.L. Shepard*, Columbia University, *R. Levicky*, Columbia University and Polytechnic University

Solid-phase hybridization underpins modern microarray and biosensor technologies. While the underlying molecular process, namely sequence-specific recognition between complementary probe and target molecules, is fairly well-understood in bulk solution, this knowledge proves insufficient

to adequately understand solid-phase hybridization. Using self-assembled DNA monolayers as a model system for hybridization assays, the influence of ionic strength and probe coverage and their cross-correlation are studied systematically on mm-sized gold electrodes. Electroactive ferrocene and ruthenium compounds were employed to quantify the surface DNA probe and target densities independently. The use of electrochemical labels enables in situ monitoring of the hybridization process as well as quantification of nonspecific versus sequence-specific attachments of targets. Results of these experiments can be summarized in a hybridization "map" as a function of ionic strength and probe coverage. Optimum probe densities that lead to maximum target binding or, alternately, maximum hybridization efficiency under a given set of conditions have been identified. The objective is to obtain better understanding of the physical characteristics of solid-phase hybridization at a more fundamental level and to subsequently use this knowledge to guide DNA microarray and other surface hybridization applications.

3:00pm **BI-WeA5 Hybridization with DNA Probes Bound to Gold by Adenine Nucleotides***, **A. Opdahl**, D.F. Shudy,* University of Wisconsin, La Crosse*, L.J. Whitman,* Naval Research Laboratory*, D.Y. Petrovykh*, University of Maryland, College Park, and Naval Research Laboratory

The surface density of immobilized nucleotide probes is a key variable in most applications of DNA-functionalized surfaces because the inter-molecular spacing has a strong impact on subsequent hybridization. In an earlier work, it was demonstrated that probe spacing can be controlled by exploiting the strong and preferential interaction between oligo(dA) and gold.[1] Using a model set of d(T_m-A_n) oligos, the lateral spacing between DNA molecules was found to be largely determined by the number of nucleotides, n, in the (dA) component; e.g. increasing the number of dA nucleotides in the sequence increased the spacing between probe strands. Here, we use both in situ (SPR) and ex situ (XPS) methods to demonstrate that the surface density of realistic DNA probe sequences can be controlled on gold by incorporating a d(T_m-A_n) 'tail' in the sequence. We find that surfaces functionalized in this fashion possess many desirable properties, including simplicity in fabrication, highly reproducible hybridization kinetics, and stability over multiple hybridization/melting cycles. The unique feature of our strategy is the relationship between the probe spacing and the length of the dA component in the probe. We find that an even wider range of probe-to-probe spacing can be achieved by co-immobilizing the probe DNA with unmodified oligo(dA), which acts as a lateral spacer. Altering either the length or mole fraction of this spacer systematically changes the probe DNA surface coverage, and thereby systematically modulates the hybridization response. Finally, we will discuss how hybridization with probes immobilized via our approach compares with DNA probes on gold prepared by more conventional strategies.

[1]Opdahl et al., Proc. Nat. Acad. Sci., 104, 9, (2007).

4:00pm **BI-WeA8 Novel Materials and Strategies for DNA Sequencing and Genotyping in Microfluidic Devices***, **A.E. Barron**,* Stanford University*, C.P. Fredlake,* Northwestern University*, J.A. Coyne,* Stanford University*, J.S. Lin, R.J. Meagher*, Northwestern University **INVITED**

High-resolution DNA separations are necessary for electrophoretic DNA sequencing and genotyping, which remains an extremely important workhorse technology even in the present, so-called "post-genomic era". In currently used capillary electrophoresis instruments, polymer networks provide the required molecular sieving of DNA fragments. Electrophoresis in sieving matrices has intrinsic physical limitations in read length, shows reduced performance under high electric fields, and requires capillary loading with viscous polymer solutions. There is an ongoing push toward performing DNA sequencing in miniaturized "lab-on-a-chip" devices, which promise higher throughput and lower cost. We are developing "End-Labeled Free-Solution Electrophoresis" (ELFSE) as way to separate DNA according to size without the need for a sieving matrix. In ELFSE, each DNA molecule in a sample is covalently modified with a unique frictional modifier or "drag-tag" that modifies DNA electrophoretic mobility in a size-dependent fashion. We have designed and synthesized a series of non-natural polypeptide and polypeptoid drag-tags, and conjugated them to DNA for free-solution separations by microchannel electrophoresis. In one approach, artificial genes encoding repetitive polypeptides are constructed by controlled cloning, expressed in E. Coli, and purified. These protein drag-tags have so far have been used to demonstrate 4-color sequencing of ~ 180 bases of DNA by capillary electrophoresis, in the absence of a sieving matrix. The obtainment of longer drag-tags, so that we can get longer reads, is ongoing, and sequencing separations are now being carried out in glass microfluidic chips. The application of hydrophilic polymer wall coatings are a critical element of making this technology work, and this will be discussed in some detail. ELFSE may be the a breakthrough that enables rapid, high-throughput sequencing in integrated microfluidic devices, with all of the accompanying advantages that chips offer.

4:40pm **BI-WeA10 Multivariate Analysis Methods Applied to ToF-SIMS Images of DNA Microarrays***, **P.-C. Nguyen**, L.J. Gamble,* University of Washington*, C.-Y. Lee,* 3M Corporate Research Analytical Laboratory*, G.M. Harbers, B.J. Tyler, D.W. Grainger,* University of Utah*, D.G. Castner*, University of Washington

The printing process for preparing DNA microarrays introduces variability in microspots, as observed with fluorescence detection commonly used to analyze these arrays. The nanolitre drops of solution printed onto the microarray surface dries within seconds. During this rapid drying the solution ionic strength and solute concentrations increase dramatically. Interspot variations and non-uniform distribution of probe molecules within spots are major sources of experimental uncertainty in microarray analysis. Various primary ion sources (Bi^+, Bi_3^+, Bi_3^{++}, C_{60}^+, C_{60}^{++} and C_{60}^{+++}) were used in imaging time-of-flight secondary ion mass spectrometry (ToF-SIMS) to study this non-uniformity. The type of information gained from using different primary ion beams is compared. Principal component analysis (PCA) and maximum autocorrelation factors (MAF) were used to analyze the image results and determine which masses were the main causes of the observed variability. Amine-modified single-stranded DNA was immobilized on commercial slides containing NHS groups. Spots containing 0 to 100% of fluorescent Cy3 labeled DNA were examined. Different percentages of Cy3 label resulted in variations in spot size and shape as well as differences in fluorescence distribution within spots. Imaging ToF-SIMS showed that additives in the print solution (sodium dodecyl sulfate, N-lauroyl sarcosine, salts, etc.) as well as the Cy3 labeled DNA were non-uniformly distributed within the microspots. These non-uniformities were more apparent in images acquired with Bi_3^+ and Bi_3^{++} compared to images acquired with Bi^+. Compared to univariate analysis (i.e., examination of individual masses), both PCA and MAF methods more readily highlighted the distributions of chemical non-uniformities present in the DNA microspots. Results thus far indicate that most detail about types and distribution of chemical species in DNA microspots have been obtained from MAF analysis of the Bi_3^{++} images. However, C_{60}^{++}, C_{60}^{+++} data is providing additional information that is been examined.

5:00pm **BI-WeA11 Quantitative Analysis of Block-oligonucleotide Brushes on Gold***, **D.Y. Petrovykh**,* University of Maryland, College Park, and Naval Research Laboratory*, A. Opdahl,* University of Wisconsin*, L.J. Whitman*, Naval Research Laboratory

DNA brushes with unique properties can be prepared using a new immobilization method that is based on the intrinsic affinity of blocks of adenine nucleotides for gold.[1] Block-oligonucleotides are single-stranded DNA (ssDNA) with sequences that follow, for example, a d(A_k-T_m-N_n) pattern: a block of k adenine nucleotides [d(A_k)], followed by a block of m thymine nucleotides [d(T_m)], and a short sequence of n (arbitrary) nucleotides [d(N_n)]. Brushes formed by the d(A_k-T_m-N_n) block-oligonucleotides are particularly interesting, because they attach to gold via the d(A) blocks and present the d(N) "probe" sequence for hybridization with complementary nucleotides. We are quantitatively characterizing these DNA brushes before, after, and during the hybridization experiments using a combination of x-ray photoelectron spectroscopy (XPS) and surface plasmon resonance (SPR) imaging. Characterizing these systems presents several analytical challenges. First, unambiguous deconvolution of XPS spectral features is not possible for these chemically non-uniform brushes. Second, the low surface density of the d(N) probe sequences, which is required to maintain high hybridization efficiency, results in low XPS intensities. Finally, DNA hybridization is carried out in an aqueous environment, so comprehensive analysis of these experiments requires establishing quantitative correlations between the in situ SPR imaging and ex situ XPS measurements. We addressed these challenges by taking advantage of the high degree of control over the composition and grafting density of ssDNA brushes immobilized via adenine blocks. The resulting sets of samples having systematically varied properties allowed us to observe and quantify by both XPS and SPR the effects of the surface density of ssDNA probes on the resulting hybridization efficiencies. Developing such quantitative methods that combine results of in situ and ex situ analysis is critical for extending well-developed surface analysis techniques to complex biological surfaces and interfaces.

[1]Opdahl, et al., Proc. Natl. Acad. Sci. USA 104, 9 (2007).

Nanometer-scale Science and Technology

Room: 615 - Session NS1+BI-WeA

Biological and Molecular Applications of Nanoscale Structures

Moderator: J. Randall, Zyvex Corporation

1:40pm **NS1+BI-WeA1 Multiplexed DNA and Protein Arrays Printed via Dip Pen Nanolithography**, ***N.A. Amro***, *S. Rozhok, T. Renner, J. Fragala, M. Nelson*, NanoInk, Inc.

Miniaturized nucleic acid in the form of nanoarrays will dramatically enhance the sensitivity, and spatial density of chip-based bio-assays. These nanoarrays will improve applications ranging from point-of-care diagnosis to genomic arrays used in basic research by enabling the development of next generation screening technologies that are faster, more sensitive, more reliable, and possibly more cost effective than those presently available in the life sciences market. Nucleic acid bioarrays can be printed using Dip-Pen Nanolithography® (DPN®), a new direct-write spotting technology which generates sub-micron sized features of DNA or protein on solid surfaces. This printing technique offers significant advantages over current microarray printing technologies that suffer from poor spot to spot reproducibility in terms of size, shape, and oligonucleotides density, as well as reproducibility across microarray slides. In this talk we report on recent technical advances in patterning DNA and protein patches with submicron dimensions on glass and metal coated substrates, using new commercially available DPN accessories such as multiple pen arrays and microfluidic pen loading which allows performing multiplexed DNA and protein patterning to fabricate arrays with nanoscale registration and high throughput printing. DNA and protein dot features as small as 200 nm in diameter can be routinely generated. DPN patterning of proteins and oligos, as well as screening for their biological activity, will be shown and discussed in detail.

2:00pm **NS1+BI-WeA2 Cell-Surface Interactions: The Extracellular Matrix as Mechanotransducer**, ***V. Vogel***, ETH Zürich, Switzerland **INVITED**

While cells initially respond to the surface chemistry presented on synthetic materials, they rapidly begin to assemble their own matrix. Cells can thereby sense and transduce a broad range of mechanical forces into distinct sets of biochemical signals that ultimately regulate cellular processes, including adhesion, proliferation, differentiation, and apoptosis. But how is force translated at the molecular level into biochemical signal changes that have the potential to alter cellular behavior? The molecular mechanism of fibronectin's (Fn) extensibility within extracellular matrix fibrils is controversial. Does it originate from the force-induced extension of a compactly folded quaternary structure, or from unfolding of fibronectin modules? Clarification of this issue is central to our understanding whether or not the extracellular matrix can act as a mechanotransducer that converts mechanical forces into well regulated biochemical signal changes. Different fluorescence resonance energy transfer (FRET) labeling schemes were used to differentiate between these two models and we quantified how the conformational changes of fibronectin probed by FRET relate to changes of its overall end-to-end extension. The data clearly demonstrate that cells do indeed mechanically unfold fibronectin. The functional implications of the findings are discussed as well as high resolution structural models derived from steered molecular dynamics (SMD) how force might change the functional states of this and other multidomain proteins.

2:40pm **NS1+BI-WeA4 Carbon Nanotube - Chitosan Sites for Direct Electrical Sensing of Biomolecular Events in BioMEMS**, ***S.L. Buckhout-White***, *S.K. Gupta, M.S. Fuhrer, G.W. Rubloff*, University of Maryland

Direct electrical sensing of biomolecular events is highly desirable in bioMEMS applications such as metabolic engineering platforms or biosensor systems. We are pursuing the development and integration of carbon nanotube (CNT) thin film biosensing devices in the microfluidic systems we employ for recreating metabolic pathways for drug discovery. Since these systems exploit chitosan electrodeposition as a spatiotemporally programmable reaction site for biomolecular binding and enzymatic activity, CNT biofunctionalization using chitosan would enable fabrication of the microfluidics environment with embedded CNT devices so that the devices could serve as active sites for biomolecular events and simultaneously read out those events electrically. We have developed integrated CNT devices, for use as conductance elements and/or FET's, and an easily fabricated, reusable microfluidic system using SU8 channels and a PDMS gasket for non-permanent sealing. In addition, we have electrodeposited chitosan, an amine-rich polysaccharide, onto CNT mats exposed as active sites for biomolecular binding down to 1 µm resolution. Since we have already demonstrated that proteins,[1] nucleic acids, and viruses[2] can be conjugated to localized chitosan sites in bioMEMS and retain their biomolecular functionality, we believe the integrated CNT devices are promising for sensing and controlling biomolecular assembly in multi-site bioMEMS networks that recreate metabolic pathways. This work is supported in part by the Robert W. Deutsch Foundation.

[1]Park, J.J., et al., Chitosan-mediated in situ biomolecule assembly in completely packaged microfluidic devices. Lab on a Chip, 2006. 6(10): p. 1315-1321.

[2]Yi, H.M., et al., Patterned assembly of genetically modified viral nanotemplates via nucleic acid hybridization. Nano Letters, 2005. 5(10): p. 1931-1936.

3:00pm **NS1+BI-WeA5 The Study of FET Flow Control and Separation of Proteins in Nanofluidic Channels**, ***Y.-J. Oh***, University of New Mexico, *D. Bottenus*, Washington State University, *D.N. Petsev*, University of New Mexico, *C.F. Ivory*, Washington State University, *S.R.J. Brueck, G.P. Lopez, S.M. Han*, University of New Mexico

We have studied field-effect-transistor (FET) flow control and separation of proteins in a parallel array of nanochannels (100 nm W ×500 nm D), using scanning laser confocal fluorescence microscopy (SL-CFM) and multiple internal reflection Fourier transform infrared spectroscopy (MIR-FTIRS). For fluidic FET, a DC potential is applied to the gate surrounding an isolated mid-section of the channels under longitudinal electric field along the nanochannels. The gate potential controls the surface charge on SiO_2 channel walls and therefore the ξ-potential. Depending on the polarity and magnitude, the gate potential can accelerate, decelerate, or reverse the flow of proteins. We also analyze a pH shift in the nanochannels according to the surface charge modulation and longitudinal electrical field, using Fluorescein as a pH indicator. Our MIR-FTIR analysis shows that Fluorescein dye molecules are hydrogenated and dehydrogenated in response to the gate bias and subsequent pH shift. We demonstrate that the pH shift affects the FET flow control with SL-CFM analysis. A nanochannel device containing multiple gates is used to improve the controllability of protein flow and to introduce a pH gradient along the channels for isoelectric focusing. A different potential is applied to each gate to differentially control the surface charge on the SiO_2 channel walls and to create a pH gradient along the channels. We also generate a pH gradient along the nanochannels, induced by controlled water electrolysis under longitudinal electrical field. The control and separation of proteins, which have different isoelectric points (pI), by the pH gradient along the nanochannels as a function of gate bias and longitudinal electrical field will be further discussed in this presentation.

4:00pm **NS1+BI-WeA8 Microfabricated Cantilever Arrays for Drug Screening Based on DNA-Drug Intercalation**, ***R. Desikan***, Oak Ridge Associated Universities, *H.M. Meyer III, T.G. Thundat*, Oak Ridge National Laboratory

The ability of a cantilever sensor to generate nanomechanical motion from biomolecular interactions can have wide applications in drug discovery. Microfabricated cantilever arrays offer high sensitivity multiplexed detection in real-time in liquid medium. DNA strands when selectively immobilized on one side of the cantilever surface exhibit surface stress variation during interaction with different chemicals. We have used microfabricated piezoresistive cantilever arrays to demonstrate surface stress variation due to immobilization of ssDNA, hybridization and drug interaction that could form the basis for multiplexed detection of chemicals and drugs intercalating with dsDNA. It is shown that immobilization of ssDNA results in a compressive stress while hybridization results in tensile stress. Intercalation between adjacent base pairs results in stiffening, lengthening and unwinding of the double helix, causing it to expand and lose native conformation. Intercalation of chemicals into immobilized dsDNA produces a characteristic oscillatory response pattern. We have investigated the surface stress pattern due to interaction of chemicals such as ethidium bromide and anti-tumor drugs with dsDNA immobilized on cantilever arrays. The dynamic signature pattern can provide new insight on the kinetics and nanomechanics of DNA-drug intercalation. We have used x-ray photoelectron spectroscopy to carry out elemental analysis in order to confirm the presence of the intercalating agent in the DNA. Microfabricated cantilever arrays find potential applications in drug screening, identification of various toxins, and biological sensing.

4:20pm **NS1+BI-WeA9 Nanofluidic System for Investigating DNA-Force and DNA-Protein Interactions**, ***V.R. Dukkipati***, *S.W. Pang*, University of Michigan

Nanofluidic systems are useful in the studies of single-molecule DNA-protein interactions, nanofluidics, and polymer dynamics. DNA is a flexible polymer with a large aspect ratio of 2 nm in width and length in the order of the micrometers. Due to its unique properties, it is ideally suited for investigations using nanofluidic systems for both biological and mechanical characteristics. We present an integrated nanofluidic system, which is

applied to study DNA-protein interactions and DNA-force dynamics. In this system, electrodes are integrated in sealed Si nanochannels by PMMA bonding. Cr/Au electrodes are patterned on a 100 µm thick glass followed by PMMA coating and patterning to expose the electrodes. The 100 µm thick glass allows high resolution imaging of DNA at single molecule level. Si nanochannels varying from 350-500 nm in width are sealed with electrodes using PMMA bonding. A 150 µm wide, 1 µm deep microchannel is connected to each of the fluidic ports as an interface to the nanochannels. The microchannel allows the DNA molecules from the inlet port to have an easy access to the nanochannels. The DNA molecules are pumped into the nanochannel array using both hydrodynamic force and electric field. Using the hydrodynamic force, the DNA molecules near the inlet are pumped by evaporation from the outlet port, which is open to atmosphere. Using 100-300 KHz electric field generated by ac voltage applied across the electrodes integrated in the microchannel, the DNA molecules are driven from the microchannel into the nanochannel array. The linear motion generated by the electric field causes the DNA molecules to move in the nanochannels with velocity varying from 5-40 µm/s. This corresponds to a viscous drag force of 0.04-3.80 pN acting on the DNA molecules, assuming the values of viscosity of the water and the radius of the DNA molecules of 0.5 µm in nanochannels. The nanofluidic system is also used to immobilize and stretch T2-DNA molecules using the protein assisted DNA immobilization (PADI) technique. 350 nm wide and 100 nm deep channels are used to immobilize and stretch λ-DNA molecules up to 12 µm long. Further studies will be conducted on the influence of multiple sources of electric field on DNAs in nanochannels.

4:40pm **NS1+BI-WeA10 Plasma Assisted Production of Chemical Nano-Patterns: Study of the Biosensing Efficiency**, *A. Valsesia,* ***P. Colpo****, I. Mannelli, P. Lisboa, F. Bretagnol, G. Ceccone, F. Rossi*, European Commission - Joint Research Centre, Italy

The next challenge for the development of analytical devices for biological analysis relies on the ability to design advanced surfaces able to interact properly with the biological world. An increase of several order of magnitude of analysis capacity in biosensing devices together with lower detection limits is envisaged, due to the special interactions between the biomolecules and the nanostructured materials. The most important consequences of the nano-structuring of the bio-interacting surfaces is the immobilization of the biomolecular probes in an active state, limiting the non specific adsorption and the optimisation of their binding site accessibility for the bio-recognition of the target molecules. In our laboratory, we have developed alternative fabrication strategies for the creation of chemically nanostructured surfaces by combining Colloidal Lithography and Electron Beam Lithography with Surface Functionalization Techniques such as Plasma Enhanced Chemical Vapour Deposition (PE-CVD) of bio-functional polymers and Self Assembled Molecular Monolayers (SAM). In particular carboxylic functionalized nano-domes in a PEO-like anti-fouling matrix have been produced. We showed that these chemical nano-patterns are able to immobilize proteins selectively in the carboxylic functional nano-domains, leaving the anti-fouling matrix clear Moreover, we have compared the detection performances between uniformly functionalized surface and chemically nano-patterned surfaces when applied as platforms for antigen/antibody interactions. In particular, homogeneous PAA was compared with the PAA nano-areas in anti-fouling matrix previously described. Nano-patterned surfaces showed a considerable enhancement of the immunoreaction efficiency with respect to the non-structured surfaces, demonstrating the capability of nano-patterns to improve the binding site accessibility of the immobilized biological probes.

Biomaterial Interfaces

Room: 609 - Session BI-ThM

Biomimetic Phospholipid Interfaces

Moderator: F. Höök, Lund University, Sweden

8:00am **BI-ThM1 Surface-supported Bilayer Platforms for Fundamental Research and Biotechnological Applications**, ***K. Hristova***, Johns Hopkins University **INVITED**

Approximately 20% of the open reading frames in complex organisms encode membrane-associated proteins. Despite their abundance and key roles in cell adhesion, recognition, motility, energy production, transport of nutrients and cholesterol, our knowledge of the folding and the structure of membrane proteins is limited, and lags far behind that of soluble proteins. In part, this is due to limited biophysical tools to adequately probe the physical-chemical principles underlying membrane protein function. In our laboratory we have developed a model surface-supported bilayer platform, based on a directed assembly approach that overcomes current limitations associated with traditional black lipid membranes and self-assembled membrane mimetics. The behavior of the proteins in the surface-supported bilayer, as determined by their sequence, is not altered by the assembly method. Thus, the platform is suitable for biophysical characterization of membrane proteins and can be used as a tool to probe diffusivity, secondary structure, thermodynamics of interactions, and electrical response. The long-term goal is to use the platform for biotechnological applications, including drug screens and analyte sensing.

8:40am **BI-ThM3 Supported Lipid Structures as Model Systems for Membrane Associated Interactions**, *S. Svedhem, **A. Kunze**, E. Briand, A. Wikström, B. Seantier, P. Axelsson, H. Ekstrand, M. Edvarsson, S. Petronis, M. Zaech, B. Kasemo*, Chalmers University of Technology, Sweden

Engineering of surface-supported lipid membrane model systems is currently a very active field of research. The present contribution will present a number of recent examples in this area from our group, including both different kinds of supported lipid structures; supported lipid bilayers and vesicles, tethered vesicles, and hole-spanning membranes; as well as different kinds of biomolecular interactions associated with them. The main techniques used to follow these processes are the quartz crystal microbalance with dissipation monitoring (QCM-D), surface plasmon resonance (SPR), optical reflectometry, fluorescence microscopy, and atomic force microscopy (AFM). Some new findings about the factors influencing how lipid bilayers or monolayers of intact vesicles are formed on SiO2 surfaces, e.g. with respect to cations present in the buffer, will be presented. We will also describe new results about processes on the suported lipid structures. In particular, the action of different kinds of lipases (via hydrolysis of phospholipids) on supported lipid bilayers has been monitored by different techniques, as well as the effect of lipases on tethered vesicles (including PEG-ylated vesicles of relevance for drug delivery applications). Another example that will be covered is the exchange of lipid material between charged supported lipid membranes and vesicles; potentially a method for the in situ modification of supported membranes.

9:00am **BI-ThM4 Anisotropic Diffusion in Nanopatterned Supported Lipid Bilayers**, ***J. Tsai***, *E. Sun, J.C. Hone, L. Kam*, Columbia University

Membrane-associated proteins have a central role in cell-cell adhesion and communication, mediated in part by the ability of membrane proteins to diffuse along the cell surface. Many of these proteins exhibit long-range (tens of micrometers) diffusion coefficients that are orders of magnitude smaller than that expected for membrane components. Moreover, other experiments suggest that over short (submicrometer) distances, the diffusive properties of these proteins more closely resemble that of membrane lipids. To capture this anomalous diffusion in a controllable, in vitro model, glass-supported lipid bilayers were patterned with nanoscale barriers of chromium and/or titanium, creating periodic barriers that mimic the spacing of cytoskeletal elements in cells (which underlie several models of anomalous diffusion). Specifically, these barriers consisted of 50-nm wide, parallel barriers spaced at 125 and 250 nm intervals. Gaps in these barriers, measuring 30-50 nm and spaced at 500 nm intervals, were introduced to allow a limited amount of long-range diffusion across the barriers. Long-range diffusion coefficients of Texas-Red-DHPE, in a background of vesicles of Egg PC, were measured using an image-based, fluorescence recovery after photobleach approach. The long-range diffusion coefficient of lipids parallel to the barriers was similar to that on non-patterned glass for both types of metals and all geometries. In contrast, long-range diffusion perpendicular to the barriers was decreased by as much as a factor of ten, dependent on the pattern geometry. Barrier spacing, rather than gap size, was the major determinant of long-range diffusion. Barrier material had an additional influence. On surface with chromium lines, photobleach recovery agreed with a model of diffusion along a perforated surface, suggesting that this material forms perfect barriers to lipid diffusion. On surfaces patterned with titanium, diffusion across the barriers was consistently higher than predicted by the model; furthermore, lipids exhibited limited diffusion across barriers with no gaps. These results suggest a more complex interaction between the supported lipid bilayer and the substrates. The nature of this interaction is currently under investigation. In summary, we describe a controllable, nanopatterned supported lipid bilayer model that captures the complex patterns of membrane protein diffusion, which have immediate use in the study of cell-cell communication.

9:20am **BI-ThM5 Fabrication of Surface-Engineered 3D Micro-Well Arrays for High Resolution and High Throughput Analysis in a Single Cell Format**, ***M. Textor***, *M. Ochsner, M. Smith, H.M. Grandin, S. Luna-Morris, V. Vogel*, ETH Zurich, Switzerland

In addition to substrate rigidity, matrix composition, and cell shape, dimensionality is now considered an important physical property of the cell microenvironment which directs cell behavior. However, available tools for the study of cell behavior in two-dimensional (2D) versus three-dimensional (3D) environments are difficult to compare, and no tools are available which provide 3D shape control of individual cells. Using microfabrication and replication techniques, we developed PDMS substrates for the culture of single cells in 3D arrays compatible with both high-resolution microscopy and high-throughput analysis. Cell adhesion was limited to within microwells by passivation of the flat upper surface through 'wet-printing' of a non-fouling polymer and backfilling of the wells with either specific adhesive proteins or lipid bilayers, the latter produced by exposing the oxidized PDMS wells to lipidic vesicles that spontaneously fused to form supported lipidic membranes. The surfaces were characterized at each fabrication stage by confocal laser scan microscopy (CLSM) in conjunction with labeled polymers and proteins, and the Quartz Crystal Microbalance (QCM-D) technique and FRAP to characterize bilayer formation on PDMS surfaces. Endothelial cells constrained within microwells were viable, although cell death was increased in very constrained microwells as has been reported for cells on flat substrates. In contrast to studies on 2D surfaces, actin stress fibers were present even within cells cultured in very constrained microwells, and in addition the cytoskeleton was 3D and not limited to the cell-substrate interface. These observations demonstrate that microwells can be used to produce microenvironments for large numbers of single cells with 3D shape control and can be added to a repertoire of tools which are ever more sought after for both fundamental biological studies as well as cell-based assays for drug development and screening. Future work is directed towards the study of (stem) cell differentiation and its dependence on surface (bio)chemistry, ligand mobility and substrate rigidity as well as the development of microwells with walls covered by cadherin-functionalized lipidic membranes simulating a microenvironment that is possibly closer to the one of cells in multicellular colonies and tissue.

9:40am **BI-ThM6 Effects of Fluidity on HIV-1 Neutralizing Antibody Binding to Membrane Surfaces**, ***Y. Lam***, *W. Goo, S.M. Alam, S. Zauscher*, Duke University

Recent studies show that HIV-1 may take advantage of the phenomenon that healthy individuals do not normally produce self reactive antibodies. Broadly neutralizing monoclonal antibodies (nAbs) 4E10 and 2F5 bind to epitopes in the membrane proximal external region (MPER) on the HIV-1 transmembrane envelope glycoprotein (Env) gp41. Unlike most antibodies, however, they also react with several common membrane phospholipids. This auto-reactive characteristic may explain why they are rarely (if ever) found in HIV-1 patients. Little is known about the mechanics of these interactions between nAb and membrane. Initial surface plasmon resonance (SPR) studies suggest a two phase binding model where the nAb first encounters the membrane epitope proximal region, and then docks more securely in a second step. One theory for the mechanism behind this model suggests nAb may first bind to lipid, then diffuse on the lipid until encountering the MPER region on Env gp41. In our work, we investigate the effects of membrane fluidity in this process. Increasing temperature adversely affects this interaction, as it increases the on-rate, but more rapidly increases the off-rates. In order to eliminate rate effects, we monitor protein binding to peptide sequences embedded in mobile supported lipid bilayers and in static lipid monolayer surfaces. Quartz crystal microbalance with dissipation monitoring (QCM-D) was used to verify bilayer and

monolayer formation as well as to measure nAb binding. This knowledge will provide insight into the mechanism of nAb-lipid binding, which may facilitate the production of effective HIV-1 therapies.

10:00am **BI-ThM7 Rupture, Spreading, and Healing of 2D Fluid Lipid Bilayers at Chemically and Topographically Structured Surfaces**, ***A. Parikh***, University of California, Davis **INVITED**

Interfacial organization of lipids and amphiphiles into a discrete number of molecular layers provides, arguably, one of the most pristine experimental realizations of self-organized, two-dimensional systems. it provides an experimental test-bed for the study of a rich variety of interface-dominated processes, including surface melting, low-dimensional phase transitions, surface dynamics, and phase coexistence and separation. This talk will present recent experimental evidence from our laboratories which highlight the importance of substrate structure (e.g., topography, charge, and surface energies) in influencing the dynamics of formation of interfacial single lipid bilayers and their equilibrium morphologies. This work is performed in collaboration with Babak Sanii, Michelle Smith, Alan Szmodis, Adrian Brozell, and Michael Howland. The work is supported by a grant from U.S. Department of Energy.

10:40am **BI-ThM9 Activity of Transmembrane Protein Maintained in Polymerized Lipid Membranes**, ***H. Zhang***, University of Arizona, *V. Subramaniam*, Univeristy of Arizona, *S. Burnside, G. D'Ambruoso, M. Brown, S. Saavedra*, University of Arizona

Membrane-associated proteins are of great importance in transport and signal transduction cascades and therefore are crucial pharmacological targets. With the goal of developing biosensors to screen pharmaceutical candidates, numerous research groups have reconstituted transmembrane proteins into lipid membranes, which provide a biocompatible environment. Our group has been investigating the use of synthetic lipids that can be polymerized to form a highly stable bilayer. Absorbance spectroscopy and plasmon waveguide spectroscopy (PWR) are being utilized to monitor the photoactivity of a G-protein coupled receptor (GPCR), bovine rhodopsin, in both polymerized liposomes and planar supported lipid bilayers (PLSBs), respectively. The photoactivity of rhodopsin is largely retained in some types of poly(lipid) membranes. However, the lipid structure and bilayer polymerization clearly affect the retention of protein activity. PWR is used to probe conformational changes accompanying rhodopsin photoactivation in PSLBs, whereas absorbance spectroscopy is used to directly measure formation of the rhodopsin activated state. Studies are also being performed to investigate the effect of lipid structure on the activity of other types of GPCRs, e.g. the human delta opioid receptor.

Tribology

Room: 617 - Session TR2+BI+NS+MN-ThM

Biolubrication, Sensing and Adhesion

Moderator: R.W. Carpick, University of Pennsylvania

8:00am **TR2+BI+NS+MN-ThM1 Resonant Nanomechanical Sensors for Protein Detection**, ***P.S. Waggoner***, *H.G. Craighead*, Cornell University

Micro- and nanoelectromechanical systems (MEMS and NEMS) are of interest in sensing applications due to their high sensitivity, label-free operation, and potential for mutilplexed detection on a single chip. Resonant MEMS and NEMS devices have demonstrated detection of masses on the order of femtograms or less, transducing changes in mass into changes in resonant frequency. Appropriate functionalization of the sensor surface allows specific, label free detection for analytes of choice. In this work we have detected prostate specific antigen (PSA), a biomarker used in the early detection of prostate cancer, as a model system using immunospecific functional layers present on the resonator surfaces. We have also studied the surface chemistry in order to minimize non-specific binding during sensor functionalization and use. In addition, sandwich assay techniques have been investigated for use in secondary mass tagging in order to enhance sensor response for dilute analytes while still preserving specificity.

8:20am **TR2+BI+NS+MN-ThM2 Correlation between XPS Data and Liquid Phase Self-Assembly of Alkanethiols**, ***H.M. Meyer III***, *T.G. Thundat, R. Desikan*, Oak Ridge National Laboratory, *R.G. White*, Thermo Fisher Scientific, UK

The relative ease in which self-assembled monolayers (SAM) can be applied have made them part of the standard tool set used for functionalizing and patterning surfaces at the nanoscale. Recently, alkanethiol-based SAMs have been used for immobilizing selective chemical receptors on the gold-coated side of a microcantilever. In this configuration, adsorption on the funtionalized side of the microcantilever generates nanomechanical motion (i.e. bending) which can be accurately sensed and used for detecting a variety of chemical and biological molecules. Achieving reliable selectivity and sensitivity depends primarily on the reproducible formation of the functional layer on one side of the microcantilever. We have recently investigated the effect of chain length on the packing density of the alkanethiols and, in turn, how this affects the sensitivity of the sensor. We present XPS characterization of microfabricated cantilevers functionalized with alkanethiol-based SAMs. The results are correlated with similar cantilevers that have been monitored during adsorption/immobilization of the same alkanethiols in the liquid phase. Previous liquid phase results indicated an unusual change in packing density of the thiol molecules as the chain length was increased and were difficult to correlate with preliminary XPS data, indicating major difference between how these films form in liquid phase vs. post-formation analysis in-vacuuo. These new results attempt to illuminate those differences. Research sponsored in part by grant NSF Award ID 0330410 in collaboration with Drs. V.P. Dravid, G. Shekhawat, and A. Majumdar and in part by the Assistant Secretary for Energy Efficiency and Renewable Energy, Office of FreedomCAR and Vehicle Technologies, as part of the High Temperature Materials Laboratory User Program, Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725.

8:40am **TR2+BI+NS+MN-ThM3 Surface-Chemical Aspects of Implant Biotribology and Biomimetic Lubrication**, ***N.D. Spencer***, ETH Zurich, Switzerland **INVITED**

Following implantation of a hip prosthesis, the synovial membrane reforms and generates a liquid, pseudo synovial fluid (PSF), which is comparable in composition to synovial fluid itself. This complex solution of proteins, glycoproteins, polysaccharides and lipids is then responsible for the lubrication of the implanted joint. Surprisingly, the interactions of PSF components with the surfaces of joint materials, such as UHMWPE, CoCrMo, or alumina, remain remarkably unexplored. Not only is their relative propensity for adsorption unknown, but, once adsorbed, their efficacy for lubrication remains to be determined. We have investigated these issues by means of tribometry (on both macro and nano scales), combined with fluorescence microscopy, and determined, for example, that while albumin, the major component of PSF and the model protein used for implant testing, is strongly adsorbed on the surface of implants, it can be displaced during sliding by Î±-glycoprotein, a species present at much lower concentrations, but a much more effective lubricant. The fluorescence approach has also enabled us to detect transfer of polyethylene under conditions where it was not previously thought to occur. Natural lubricated surfaces tend to be soft and covered with species such as polysaccharides that have the ability to retain large amounts of water. We have attempted to imitate and understand this mechanism of lubrication by means of tethering various highly hydrated polymer chains in a brush-like structure onto both hard and soft surfaces and measuring their tribological properties. Interestingly, on soft surfaces, the effect of chain tethering seems to be to effectively eliminate boundary lubrication entirely. Fluid-film-like behavior is thought to persist to very low sliding speeds, by virtue of water retention in a thin layer between the brush-covered surfaces.

9:20am **TR2+BI+NS+MN-ThM5 In-situ Measurement of Boundary-Lubrication on Articular Cartilage Surfaces**, *J.M. Coles,* Duke University, *G.D. Jay,* Brown University, *F. Guilak,* ***S. Zauscher***, Duke University

The diarthrodial (synovial) joints of the body enable locomotion and activity while withstanding millions of cycles of loading at several times body weight. Recent macroscopic tribological experiments and biochemical analyses suggest that heavily glycosylated proteoglycans encoded by the gene proteoglycan 4 (Prg4), which are expressed by synoviocytes in synovial fluid as lubricin and by superficial zone chondrocytes of articular cartilage as surface zone protein (SZP), provide boundary lubrication in cartilage in the absence of interstitial fluid pressurization. Improved understanding of the role of Prg4 on the cartilage surface could thus provide important insight into the development of new therapies for joint diseases such as OA. The development of powerful new methods for the genetic manipulation of mice has led to the creation of modified murine strains in which specific gene inactivation (PRG4-/-) results in age-related joint

degeneration that recapitulates the symptoms of OA. Here we show that atomic force microscopy with a colloidal probe is uniquely suited to study boundary lubrication of murine cartilage in-situ and in absence of other lubrication mechanisms. Here we report on friction measurements on the superficial surface layer of articular cartilage from the femoral head of Prg4 knockout and wildtype mice under boundary lubrication conditions. Furthermore, we report on the measured RMS roughness and Young's modulus to quantify morphological and mechanical changes of the cartilage superficial zone induced by the absence of Prg4. Our measurements suggest that the absence of Prg4 leads to increased friction, as well as degradation of the mechanical and topographical properties of cartilage. We propose that, while lubricin plays a role as a boundary lubricant, its role in chondroprotection is equally, if not more, vital.

9:40am **TR2+BI+NS+MN-ThM6 Humidity Dependent Ordering of Water and its Effect on Adhesion and Friction between Silica Surfaces**, ***B.I. Kim**, J. Bonander*, Boise State University

Adhesion and friction related to water are major problems limiting both the fabrication and long-term use of micro-machines. Adhesion and friction between two silica surfaces were measured as a function of separation distance using interfacial force microscope (IFM) for different relative humidity (RH) between 3% - 78%. The IFM provides force-distance curves without having the "snap-to-contact" problems associated with atomic force microscopy using voltage-controlled force feedback. The measured friction force-distance curves show that the friction force is oscillatory as the separation changes below thickness of water droplet. The oscillatory period is close to the mean diameter of a water molecule. The number of oscillation increased as the relative humidity increases up to RH 60% while it decreased with humidity above RH 60%. The origin of the oscillatory feature in the "interfacial" water may come from the "solid-liquid transition" between solid (ordering) and liquid (disordering). Strong correlation between the number of oscillation and the strength of the adhesion and friction indicates that the humidity dependent adhesion and friction may be attributed to the ordered structure of water molecules between two silica surfaces.

10:00am **TR2+BI+NS+MN-ThM7 Nanomechanical Properties of Arachidic Acid Langmuir Blodgett Films**, ***G. Oncins***, University of Barcelona, Spain, *J. Torrent-Burgues,* Universitat Politecnica de Catalunya, Spain, *F. Sanz*, Universitat de Barcelona and Center of Nanobioengineering of Catalonia (IBEC), Spain

Scanning Probe Microscopies development has given biophysics the possibility to deal with the interactions arisen in biological membranes from a nanomentric point of view, revealing that van der Waals, hydrogen bonding and electrostatic interactions play a crucial role in the membrane cohesion. Unfortunately, although interesting experimental conclusions have been reported in the past, these systems are complex and difficult to study.[1] In order to isolate the effect of the different cohesive interactions, Langmuir-Blodgett (LB) fatty acid monolayers provide excellent model systems because of the controlled area per molecule, linear hydrocarbon chain geometry, amphiphilic nature, high mechanical stability and the possibility to test solid and liquid phases at room temperature. The nanomechanical properties of arachidic acid LB films extracted at surface pressures of 1, 15 and 35 mN/m and deposited on mica are investigated by Atomic Force Microscopy, Force Spectroscopy and Friction Force Microscopy. It is experimentally demonstrated that the molecular ordering depends on the extraction pressure, while discrete molecular tilting angles of 50°, 34° and 22° are detected and identified as conformations that maximize van der Waals interactions between alkyl chains. The vertical force (F_v) needed to puncture the monolayer strongly depends on the molecular tilting angle, ranging from 13.07±3.24 nN at 1 mN/m to 22.94±5.49 nN at 35 mN/m. The friction force (F_f) measurements performed from low Fv until monolayer disruption reveal three friction regimes corresponding with a low F_f elastic monolayer deformation at low F_v, followed by a sharp increase in F_f due to a sudden plastic deformation of the monolayer. The last regime corresponds with the monolayer rupture and the contact between tip and substrate. Interestingly, as the extraction pressure increases, the friction coefficient of the monolayer reduces while the F_v needed to trigger the monolayer plastic deformation increases, facts that are discussed in terms of sample compactness and monolayer rupture mechanism.[2]

[1] Garcia-Manyes, S.; Oncins, G.; Sanz, F. Biophys. J. 2005, 89, 1812.
[2] Oncins, G.; Garcia-Manyes, S.; Sanz, F. Langmuir 2005, 21, 7373.

10:20am **TR2+BI+NS+MN-ThM8 Optimal Roughness for Minimal Adhesion**, *D.L. Liu,* Worcester Polytechnic Institute, *J. Martin,* Analog Devices Inc., ***N.A. Burnham***, Worcester Polytechnic Institute

Differing views on the effect of surface roughness on adhesion have appeared in the literature recently. Molecular dynamics has been used to simulate the contact of two surfaces and found that atomic-scale roughness can have a large influence on adhesion, causing the breakdown of continuum mechanics models.[1] An experimental study showed that roughness can determine the adhesion in nanometer contacts and indicated that continuum mechanics still works down to nanometer length scales.[2] In this work, we use a single-asperity model to describe a smooth tip in contact with a rough surface and predict that there is an optimal size of asperity that will yield a minimum of adhesion. Experimentally, adhesive forces on silicon wafers with varying roughness from 0.2 nm to 39 nm were measured using AFM (atomic force microscope) cantilevers with varying tip radii ranging from 75 nm to 9.08 um. It is found that for all tip radii, the adhesion falls significantly for roughness greater than 1-2 nm and drops at higher roughness for larger tips. Minimum adhesion was observed as predicted in the 1-10 nm range and the optimal roughness for minimal adhesion increases as the tip radius increases, which is also consistent with our predictions. The work presented here should help minimize adhesion in future MEMS devices and progress the understanding of adhesion between the atomic- and macro-scale.

[1] B. Luan and M.O. Robbins, Nature 435, 929-932 (2005).
[2] E.J.Thoreson, J. Martin, N.A. Burnham, J. Colloid Interface Sci. 298, 94-101 (2006).

10:40am **TR2+BI+NS+MN-ThM9 A Tribological Study of Bound plus Mobile Lubricants for MEMS Application from the Nano- to the Macro-scale Regime**, ***B.P. Miller**, M. Brukman,* North Carolina State University, *C.C. Baker,* Naval Research Laboratory, *R.J. Nemanich,* North Carolina State University, *K.J. Wahl,* Naval Research Laboratory, *J. Krim*, North Carolina State University

One possible solution to the in-use stiction problem in Micro-Electro-Mechanical systems (MEMS) is the introduction of a bound plus mobile lubricant combination.[1] For this system, one monolayer of the bound lubricant (perfluorodecyltrichlorosilane, PFTS) bonds to the surface of the device. The mobile lubricant (tricresyl phosphate, TCP) can replenish the layer after rubbing contacts wear it away, thus keeping the device free of contamination. Friction behavior of this lubricant combination was examined over three different regimes using a macroscopic tribometer, an AFM, and a quartz crystal microbalance (QCM). A comparative study of bound only versus bound plus mobile lubricants showed a decrease of the coefficient of friction with the addition of the mobile lubricant to the bound layer. Dynamic properties of the mobile film were characterized with the QCM. This work is funded by AFOSR Extreme Friction MURI Grant #FA9550-04-1-0381.

[1] W. Neeyakorn, M. Varma, C. Jaye, J. E. Burnette, S.M. Lee, R. J. Nemanich, C. Grant, J. Krim, Dynamics of Vapor-Phase Organophosphates on Silicon and OTS, Tribology Letters, in press.

Biomaterial Interfaces

Room: 609 - Session BI+AS+NS-ThA

Surface Analysis and Related Methods for Biological Materials

Moderator: S.L. McArthur, University of Sheffield, UK

2:00pm **BI+AS+NS-ThA1 Creating and Probing Model Biological Membranes**, ***S.G. Boxer***, Stanford University **INVITED**

During the past few years, our lab has developed a wide range of methods for patterning lipid bilayers on solid supports.[1] These 2D fluids are interesting both as a model for biological membranes and as a physical system with unusual properties. Methods have been developed for controlling the composition of patterned membrane corrals by variations on microcontact printing and microfluidics. Charged components can be moved around within these fluid surfaces by a form of 2D electrophoresis. The planar geometry of supported bilayer systems is ideal for high resolution imaging methods. The lateral (x-y) composition of membranes can be analyzed by high spatial resolution secondary ion mass spectrometry (SIMS) using the NanoSIMS 50 (Cameca) at the Livermore National Laboratory. Results will be described for simple membrane compositions[2] and phase separated domains[3] suggesting the potential of this method for the analysis of membrane organization in complex membranes. Extensions of this approach to more complex systems including membrane-associated proteins will be described. If time permits, a complimentary optical imaging method offering sub-nm resolution in the z-direction (perpendicular to the membrane surface) will be described in the context of imaging conformational changes in membrane proteins.

[1]J. T. Groves and S. G. Boxer, Accounts of Chemical Research, 35, 149-157 (2002).

[2]C. Galli Marxer, M. L. Kraft, P. K. Weber, I. D. Hutcheon and S. G. Boxer, Biophysical Journal, 88, 2965-2975 (2005).

[3]M. L. Kraft, P. K. Weber, M. L. Longo, I. D. Hutcheon, S. G. Boxer, Science, 313, 1948-1951 (2006).

2:40pm **BI+AS+NS-ThA3 Activation Thermodynamics for Phospholipid Flip-Flop in Planar Supported Lipid Bilayers Measured by Sum-Frequency Vibrational Spectroscopy**, ***T.C. Anglin***, *H. Li, J.C. Conboy*, University of Utah

Basic transition state theory is used to describe the activation thermodynamics for phospholipid flip-flop in planar supported lipid bilayers prepared by the Langmuir-Blodgett / Langmuir Schaeffer method. Kinetics of flip-flop are determined as a function of varying temperature and lateral surface pressure for model bilayers of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) using sum-frequency vibrational spectroscopy (SFVS). The temperature and pressure dependence of the kinetics of DSPC flip-fop provide a complete description of the activation thermodynamics for flip-flop in the gel state, including free energy of activation, area of activation, and entropy of activation. This is the first description of phospholipid flip-flop according to basic transition state theory with explicit treatment of the free-energy dependence of the process and determination of the entropic contribution to the transition state.

3:00pm **BI+AS+NS-ThA4 Observation of Electrical Characteristics at Cells Membrane using by Electrostatic Force Microscopy**, ***Y.J. Kim***, Myongji University, Korea, *H.D. Kim,* Seoul National University, Korea, *Y.S. Kim,* Myongji University, Korea, *K.H. Lee,* Seoul National University, Korea, *C.J. Kang*, Myongji University, Korea

Recent advances in atomic force microscopy (AFM) made it possible to investigate the biological materials in a single molecule level. Moreover, the AFM has been used to measure the fine structure of individual live cell even under physiological liquid. The images of cells measured show finer structure of cell boundary compared with those of SEM after fixation. Since electrostatic force microscopy (EFM) using conducting cantilever to AFM allows us to observe the electrical properties of the surface, it is also used to study the various properties of the cell membrane. Reportedly, it is known that protein expression depends on the cell kinds and shows the non uniform distributions, which causes the electrical potential difference on the cell surface in the local area. Thus mapping the electrical potential of a cell using EFM and comparing it with that of reference group, we are able to extract the information to differentiate the cells. In this work, we have identified the breast cancer cells (MCF7) and normal breast epithelial cells (MCF10A) derived from the same origin by fractal dimension analysis using AFM and the electrical properties of the cell membrane measured from the EFM will be also discussed. The results show that AFM imaging with EFM measurement might be feasible methods for analyzing surface structures of living cells with high resolution, and it could provide new insights into cell surface structure.

3:40pm **BI+AS+NS-ThA6 Two and Three Dimensional Analysis of C. Albicans Biofilms with Cluster SIMS**, ***B.J. Tyler***, *S. Rangarajan,* University of Utah*, J. Moeller, H.F. Arlinghaus*, University of Muenster, Germany

The high tolerance of microbial biofilms to important antimicrobial agents creates an import problem for treatment of infections associated with implanted medical devices. Several important hypotheses for this drug resistance involve mass transport limitation within the biofilms. We have been using 2_D and 3_D ToF-SIMS analysis to investigate transport of drugs and nutrients through C. albicans biofilms. Analysis has been performed using a novel ToF-SIMS system which incorporates a cryo-sectioning chamber and precise temperature control during analysis. This instrument has allowed us to map the distribution of key nutrients and drugs with in the biofilm as well as to identify viable vs. nonviable cells. Through these studies, we have determined that the multiple cellular layers and extracellular polymers are not the most important mass transport barrier. Common drugs are able to permeate to the bottom of the biofilm within 5 minutes but are still unable to penetrate the cell wall of persister cells found at the base of the biofilm. Current work is focusing on cell wall and membrane changes within a subpopulation of the biofilm cells which apparently limits transport of antimicrobials into the cells. Multivariate statistical techniques have been crucial for the analysis of these samples and discussion of the relevant statistical methods employed will be presented.

4:00pm **BI+AS+NS-ThA7 XPS, ToF-SIMS and NEXAFS Investigation of Peptide Adsorption onto SAMs**, ***J.S. Apte***, *L.J. Gamble, D.G. Castner*, University of Washington

The interactions between proteins and surfaces are critical to the success or failure of implants in the body. When adsorbed onto a synthetic surface, proteins often denature which can trigger the foreign-body response. It is therefore essential to develop methods to examine these interfacial phenomena. This work uses X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS) and near-edge X-ray absorption spectroscopy (NEXAFS) to characterize the structure of α-helix and β-sheet peptides adsorbed onto self-assembled monolayers (SAMs). The α-helix peptide is a 14-mer made up of lysine (K) and leucine (L) residues with a hydrophobic periodicity of 3.5. The β-sheet peptide is a 15-mer also made up of L and K residues with a hydrophobic periodicity of 2. A β-sheet peptide with the same structure but with valine (V) substituted for L was also studied since V has a higher tendency than L to form β-sheet structures. All peptides have the hydrophobic side-chains on one side of the peptide and the hydrophilic on the other. The SAMs studied were thiols on gold containing the ω-groups -CH_3, -OH, -COOH, -NH_3^+ and -CF_3. XPS nitrogen atomic percent was used to measure adsorption isotherms for the peptides. The α-helix peptide forms a monolayer (8.2% N) on the COOH-terminated SAM at an adsorption concentration 50 times lower than on the CH_3-terminated SAM (0.01 mg/mL compared to 0.5). The surface coverage on the CH_3 SAMs appeared to be patchier compared to the COOH SAMs, since they had standard deviations of 2-3% N. Atomic force microscopy images of the adsorbed peptides were generated to examine this patchiness. Also, the adsorption process depended on buffer salt concentration. Little peptide adsorption was detectable on the methyl SAMs when adsorbed from a 0.1x buffer. ToF-SIMS was used to investigate the ratio of K to L characteristic mass fragments at 84 and 86 m/z, respectively. The 84/86 (K/L) ratio on CH_3 SAMs (1.2) was the same, within experimental error, as the ratio on COOH SAMs (1.1). Polarization dependent NEXAFS experiments at the nitrogen K-edge indicated the β-sheet was lying down on the SAM surfaces. The α-helical peptide exhibited significantly less polarization dependence than the β-sheet peptide, probably due to the different structure of the backbone amide groups in the α-helical peptide.

4:20pm **BI+AS+NS-ThA8 Isolation and Detachment of Small Cell Populations from a Thermoresponsive Polymer**, ***H.E. Canavan***, *K. Gallagher-Gonzales, J.A. Reed*, University of New Mexico

Poly(N-isopropyl acrylamide) (pNIPAM) has proven to be an efficient and non-destructive means of detaching intact sheets of mammalian cells. In addition, cell sheets detached from pNIPAM maintain their association with the extracellular matrix (ECM) during and following detachment from a coated surface, enabling their use in tissue engineering. To date, the majority of those studying cellular interactions with pNIPAM have focused on harvesting large domains of cells for such tissue engineering applications. However, there are many other applications for which the non-

destructive release of smaller populations, or even isolated cells, is desirable. For example, isolated cells are required to ascertain the extent of transmembrane protein receptor upregulation when assaying the efficacy of cancer therapeutics on cell populations via flow cytometry (FC). In this work, arrays of thermoresponsive domains were fabricated to isolate defined populations of cells using a variety of techniques. The surface chemistry, thermoresponse, and topography of the films generated were verified via X-ray photoelectron spectroscopy (XPS), contact angle measurements, and atomic force microscopy (AFM), respectively, and compared to controls. The cell releasing properties of the films were characterized by incubating baby hamster kidney (BHK) and bovine aortic endothelial cells (BAECs). The behavior of the cells from isolated cells and small cell populations were characterized and compared to large cell population controls.

4:40pm **BI+AS+NS-ThA9 Surface Characterization of Ordered Nanopatterns made from Self-Assembly of Mixed Nanoparticles**, *S. Pillai, G. Singh,* The University of Aarhus, Denmark, *C. Blomfield, A. Roberts,* Kratos Analytical Ltd, UK, *R.L. Meyer,* ***P. Kingshott***, The University of Aarhus, Denmark

Controlled patterning of surfaces with different chemistries and structures at nanoscale length scales is highly desirable for understanding the fundamental mechanisms of protein and cell interactions with biomaterials. The use of nanoparticles (NPs) to pattern surfaces by colloidal lithography or templating using one type of NP is well known for applications such as biosensors,[1] biomaterials[2] and tissue engineering.[3] We demonstrate that by using mixed nanoparticles unique highly-ordered patterns can be obtained by simple self-assembly from buffer onto hydrophobic surfaces from both concentrated and dilute two-component NP suspensions.[4] The new method uses suspensions of poly(styrene) (PS) NPs of different size (d = 500 to 60nm) with different NP ratios and volume fractions. The ordering is independent of the NP surface chemistry (sulfated, carboxylated, or aminated PS) or zeta potential and occurs over a broad pH range (4-10). The method is demonstrated for two types of hydrophobic surfaces, a commercial adhesive carbon tape and octadecyltrichlorosilane (OTS)-modified glass where patterns of hexagonally packed large particles are inter-dispersed with smaller particles. However, very poor ordering is achieved using gold, mica and HOPG (highly oriented pyrolytic graphite) as substrates. The morphologies of the nanoparticle assemblies are characterised extensively by SEM and AFM. In addition, XPS and ToF-SIMS are used to characterise the surface chemistry of the NP surfaces, which also provides knowledge of the mechanisms of NP assembly by showing that the ordering is most likely associated with charge screening by buffer salts resulting in an entropically driven assembly process. These preliminary results indicate that use of two component NP assemblies opens up the possibilities of decorating surfaces with well-defined chemical nanopatterns capable of selective attachment of different proteins and/or protein resistant molecules.

[1]I. Willner, and E. Katz, Angew. Chem., Int. Ed. 2000, 39, 1180.
[2]H. Agheli , J. Malmstrom, E.M. Larsson, M. Textor, D.S. Sutherland, Nano Lett. 2006, 6, 1165.
[3]S.N. Bhatia , U.J. Balis, M.L. Yarmush, M. Toner, FASEB J. 1999, 14, 1883.
[4]R. Mukhopadhyay, O. Al-Hanbali, S. Pillai, A. Gry Hemmersam, R.L. Meyer, C.A. Hunter, K.J. Rutt, F. Besenbacher, S.M. Moghimi, P. Kingshott, submitted.

Plasma Science and Technology

Room: 607 - Session PS2+BI-ThA

Plasmas in Bioscience

Moderator: P. Favia, University of Bari, Italy

2:00pm **PS2+BI-ThA1 Time-of-Flight Secondary Ion Mass Spectrometry Analysis of Fibrinogen Adsorbed to Low-Fouling Tetraglyme Surfaces**, ***L. Mayorga***, *R. Michel, D.G. Castner, T.A. Horbett*, University of Washington

Antibody binding and ToF-SIMS were used to probe the conformation of fibrinogen (Fg) adsorbed to low and high fouling surfaces, including tetraglyme and FEP. Fg on implants plays a key role in the foreign body response (FBR) by mediating the adhesion of monocytes via the Mac-1 integrin.[1] PEO-like tetraglyme coatings generated via radio frequency glow discharge plasma display ultra-low Fg adsorption (Γ_{Fg} < 10 ng/cm^2) from low concentration blood plasma solutions and low monocyte adhesion.[2] However, subcutaneously implanted tetraglyme still exhibits FBR encapsulation. With 3 mg/ml Fg in buffer (with tracer amounts of ^{125}I-Fg added), Γ_{Fg} increased to 60 ng/cm^2 on tetraglyme and 800 ng/cm^2 on FEP. Nonetheless, the actual amount of Γ_{Fg} on glyme surfaces under any of the conditions tested is not enough to fully account for the observed monocyte adhesion in vitro. The Fg on glymes was relatively low, but adhesion was relatively high, suggesting that Fg might be in a more potent state on the glymes. To understand the role of Fg conformation in mediating monocyte adhesion, we used a monoclonal antibody to measure the degree of monocyte binding site (γ 377-395) exposure on adsorbed Fg. Epitope exposure per ng of adsorbed Fg was highest on low-fouling tetraglyme samples pre-adsorbed with low concentration Fg. In addition, ToF-SIMS was used as in previous studies[3] to characterize the conformation of Fg adsorbed to the tetraglymes. By pairing these two different approaches to study the conformation of adsorbed Fg, we will be able to relate surface analysis results with cell and protein binding data, which will allow us to better understand protein-cell interactions in the FBR.

[1]Hu W-J, Eaton JW, and Tang L. Molecular basis of biomaterial-mediated foreign body reactions. Blood 2001; 98(4): 1231-1237.
[2]Shen MC, Martinson L, et al. PEO-like plasma polymerized tetraglyme surface interactions with leukocytes and proteins: in vitro and in vivo studies. J. Biomater. Sci. Polymer Edn. 2002; 13(4): 367-390.
[3]Michel R, Pasche S, Textor M, and Caster DG. Influence of PEG Architecture on Protein Adsorption and Conformation. Langmuir 2005; 21: 12327-12332.

2:20pm **PS2+BI-ThA2 Interaction of Peptide Ions with Self-Assembled Monolayer Surfaces**, ***J. Laskin***, *O. Hadjar, P. Wang, Z. Yang*, Pacific Northwest National Laboratory

Interaction of ions with surfaces is an area of active research in surface science relevant to a broad range of other scientific disciplines such as materials science, mass spectrometry, imaging and spectroscopy. Our research is focused on fundamental understanding of interaction of hyperthermal (1-100 eV) peptide, protein and polymer ions with organic surfaces under ultrahigh vacuum conditions. Two major processes are dominant for this range of collision energies: reactive and non-reactive scattering of ions and ion loss on the surface as a result of neutralization or soft-landing (SL) of projectile ions. Scattering and deposition of large ions following collisions with SAM surfaces was studied using a unique Fourier transform ion cyclotron resonance mass spectrometer developed in our laboratory. Ion activation by collisions with surfaces is rather poorly characterized from a fundamentals perspective. We explored the effect of the physical and chemical properties of SAM surfaces on the energy transfer in collisions. Our studies demonstrated that energy distribution functions are well-represented by Maxwell-Boltzmann distributions indicating fast thermalization of ions by collisions. A notable discovery was a sharp transition between slow unimolecular decay of large ions at low collision energies and near-instantaneous decomposition (shattering) in higher energy surface collisions. Shattering of ions on surfaces opens up a variety of fragmentation pathways for large complex ions that are not accessible to conventional ion activation techniques. We have conducted first systematic study of several factors that affect SL of peptide ions on SAM surfaces. Deposition of peptide ions of different composition and charge state on SAM surfaces was followed by in situ and ex situ SIMS analysis. Peptide ions are attractive model systems that provide important insights on the behavior of soft landed proteins. We were able to measure for the first time the binding energy between peptide ions and hydrophobic SAM surfaces. We also demonstrated very strong binding of peptide ions to hydrophilic surfaces and covalent linking of peptides to reactive SAMs. Fundamental principles derived from such studies of interaction of protonated peptides with hydrophobic or hydrophilic surfaces are relevant to the understanding of the transport of biomolecules through membranes in living organisms and provides a clear pathway for highly-selective preparation of biological surfaces.

2:40pm **PS2+BI-ThA3 A New Approach to Nano-Fabrication of Functional Structures : Wet Nanotechnology and Bio Nano Process**, ***I. Yamashita***, Matsuhita Electric Industrial Co. Ltd. Japan **INVITED**

We proposed a new method for the fabrication of functional nano-structures in an aqueous solution, which could be used in semiconductor processes or electron devices and can be called a wet nanotechnology (WNT). The WNT employs aqueous solutions as the environments for nano-blocks, which are thermally agitated, to self-organize into the functional nano-structures. The final structures could be designed in the initial nano-block structures and functional nanostructures can be produced economically. This is the same with the way how the lives are carrying out in the nature. So far, we invented several processes using the WNT and proteins, which collectively we named Bio Nano Process (BNP).[1] So far, the BNP produced several key components of the electron devices. Firstly, a floating nanodots gate memory (FNGM) was produced employing a cage-shaped protein, apoferritin (collaborative project with Dr. Fuyuki at NAIST). Nanoparticles (NP) were biomineralized in the apoferritin cavity, which produced homogenous NPs such as CdSe, ZnSe, CdS, Co3O4, InOx, Fe2O3 and so on. A 2D ordered array of the apoferritins with NP was made on the Si wafer by self-assembly and heat-treated The obtained 2D ordered array of NPs was applied for the FNGM.[3] Secondly, we used 7nm Fe2O3 NPs,

which were produced and placed on Si wafer by the BNP, as the nanometric etching mask to fabricate Si single crystal nanocolumn (a collaborative project with Dr. Samukawa at Tohoku Univ). The neutral beam etching successfully produced single crystal Si columns with 7nm diameter and high aspect ratio4. We further extended the BNP application and produced a large bio-template for single electron transistor (SET).[5] A ball and spike type protein supramolecules which has a central cage-shaped protein and protruding spikes was produced by the self-assembly of genetically made chimera proteins. These experimental results demonstrated that the BNP can fabricate the inorganic nanostructure using protein supramolecules. The WNT and BNP are opening up a biological path to nano-electron devices.

[1]I. Yamashita, Thin Solid Films, 393, 12-18 (2001)
[2]K. Iwahori et. al., Biological path of nanoparticles synthesis, Dekker Encyclopedia of Nanoscience and Nanotechnology, Taylar and Francis, 1 - 8 (2006)
[3]A. Miura et. al.,Jpn. J. Appl Phys, 45(1), L1-L3 (2006)
[4]T. Kubota, et. Al., Appl. Phys. Lett., 84(9), 1555-1557, 2004
[5]K. Sugimoto et. al., Angw. Chem. Int. Edi, 45(17), 2725-2728, 2006.

3:40pm **PS2+BI-ThA6 Improvement of the Adhesion of PECVD-deposited DLC Films on Metals**, ***J.-C. Schauer**, J. Winter*, Ruhr-Universität Bochum, Germany

The coating of a material with a wear and corrosion resistant coating is required in many realms. Concerning biomedical applications for example a coating of implants made of shape memory alloys such as NiTi is needed to protect the implant against wear and corrosion and the surrounding tissue against the release of metal ions. One candidate for such a coating is a thin film of diamond like carbon (DLC, hard configuration of a-C:H), since it is very hard, wear resistant, has a low friction coefficient, is chemically inert and biocompatible. But up to the present the coating of most metals with a DLC film has shown many difficulties. The bad adhesion between substrate and DLC is due to the poor chemical binding between most metals and carbon and/or due to high internal stresses occurring in DLC films. One promising technique to overcome the problem of poor adhesion of the coating to the substrate is the deposition of a very thin interface layer on the substrate before the coating is deposited on top. By a correct material choice such an interface can replace weak coating-to-substrate bonds with strong coating-to-interface layer bonds and interface layer-to-substrate bonds. Another effect of the interface layer can be the reduction of internal stresses in the interface region. By the use of at least two layers on top of each other the probability of pinholes or defects going from the surface to the substrate is decreased. This is especially important if toxic substrates are to be coated. Therefore, we use a thin amorphous hydrogenated silicon (a-Si:H) film as an intermediate layer between metal and DLC film to enable the coating of metals with DLC films. Due to the formation of strong silicide bonds the adhesion of DLC films on metal is increased significantly. The thin films under investigation are deposited in a capacitively coupled discharge with acetylene and silane as precursor gases for the DLC and a-Si:H films, respectively. The a-Si:H films have a thickness of only several 10 nm, whereas the DLC films can have a thickness of 100 nm or more. It will be shown that an intermediate layer of a-Si:H significantly increases the adhesion of DLC on metals and how the film properties of a-Si:H and DLC influence the strength of adhesion. The influence of different parameters like applied power, substrate temperature, hydrogen content in the films, and others on the adhesion of the films also under durability tests will be presented.

4:00pm **PS2+BI-ThA7 Polymeric Surfaces Chemical Modification by Low-Pressure Plasma Processes for Application to DNA Array Technology**, ***P. Rivolo**,* Politecnico di Torino, Italy*, **S. Lo Bartolo**,* LaTEMAR, Ctr of Excellence funded by MIUR; Biodiversity SpA, Italy*, D. Perrone,* Lab. Materiali e Microsistemi, Italy; Politecnico di Torino, Italy, *S. Fiorilli,* LaTEMAR, Ctr of Excellence funded by MIUR; Politecnico di Torino, Italy*, I. Vallini,* LaTEMAR, Ctr of Excellence funded by MIUR; Biodiversity SpA, Italy*, C. Ricciardi,* LaTEMAR, Ctr of Excellence funded by MIUR; Politecnico di Torino, Italy*, M. Quaglio,* Lab. Materiali e Microsistemi, Italy; Politecnico di Torino, Italy*, G. Mantero,* Biodiversity SpA, Italy*, **C.F. Pirri***, LaTEMAR, Ctr of Excellence funded by MIUR; Politecnico di Torino, Italy

The low cost of production, the easy handling and the large variety of polymeric materials favour them as attractive candidates to replace classic glass slides in micro-array biomolecular diagnostics. However, the lack of reactive functional groups, at polymeric surfaces, makes difficult their use as substrates for immobilization of molecules such as DNA fragments, in either cDNA or oligodeoxyribonucleotide (ODN) format, for a variety of applications to DNA micro-array technology including microscale sequencing, mRNA expression monitoring and single nucleotide polymorphism analysis. In this contribution, modification of surface chemical properties of cyclo olefin copolymer (COC), polystyrene (PS), polyethylene (PE) and polycarbonate (PC) is reported. The surface of polymeric substrates, properly molded by hot embossing, was modified introducing monotype functional groups[1] and using them for subsequent covalent grafting of linker molecules, active for amino-oligonucleotide probes immobilization.[2] A first step consisting of non-equilibrium low-pressure air and water RF plasma was used to activate the polymer surface, forming oxidized species such as -C-O-, -C=O, -C-OH, -CHO, -COOH. Successively, a liquid-phase reduction by a $NaBH_4$ solution was carried out to increase the yield of –OH groups in order to enhance the amount of covalently grafted 3-aminoproylsilane (3-APTES), a reaction carried out by vapour-phase process.[3] The last step was performed by a liquid phase reaction between glutaraldehyde and amino-groups of grafted 3-APTES. After this, polymer surface shows -CHO species suitable for the reaction with the amino-modified probes. Characterization of the functionalised polymeric surfaces was performed by contact angle measurements and reflection-absorption infrared spectroscopy (RAIRS)[4]. Modification efficiency of different polymers substrates was evaluated by well-modified Arrayed Primer EXtension (APEX) protocol with colorimetric and fluorimetric detection methods.

[1] J. Friedrich, W. Unger, A. Lippitz, I. Koprinarov, A. Ghode, S. H. Geng and G. Kühn, Composite Interfaces 10(2-3) (2003) 139-171
[2] Nathalie Zammatteo et al. Anal. Biochemistry 280 (2000) 143-150
[3] W. R. Ashurst, C. Carraro, R. Maboudian, W. Frey Sensors and Actuators A 104 (2003) 213-221
[4] G.-Y. Jung et al., Langmuir 21 (2005) 1158-1161.

4:20pm **PS2+BI-ThA8 Patterning of Plasma Polymers for Bioarrays**, ***G. Mishra**, S.L. McArthur*, University of Sheffield, UK

Modern day technological advancements have allowed us to overcome critical challenges posed in proteomic research. As a direct result of developments in miniaturisation and automation, the current market has seen ever growing numbers and varieties of high density arraying slides being used for proteome research and application. Needless to say that these developments have been matched with state of art instrumentation and data analysis packages to achieve true automated multiplex analysis. Yet, issues like non-specific adsorption of biomolecules to solid substrate and control over the orientation during immobilization need addressing. Key to these issues could be the precise control over surface modification and patterning. Plasma polymerisation presents a versatile approach to surface modification of these devices. The range of monomers available for plasma polymerisation makes this manufacturing approach even more suitable for use in systems where multiple coatings with specific properties are required for a single device. The ability to spatially define reactive regions to reduce non-specific background adsorption is integral to this project. In this study we use a range of patterning techniques including photolithography and physical masks and compare the resultant pattern resolution and chemical functionality using XPS, ToF-SIMS and AFM. Plasma polymerisation when used in conjugation with photolithography has allowed us to simultaneously obtain high spatial and chemical resolution. Multivariate analysis of ToF-SIMS spectral and image data has allowed us to critically study and address issues associated with the chemical specificity and spatial resolution of the multilayer patterning approach. Our results suggest that complex multilayer plasma coatings can be produced without compromising the chemical properties of the deposited polymer layers.

4:40pm **PS2+BI-ThA9 BSA Adsorption onto Oxygen Plasma PTFE Modified Surfaces**, *B. Broze, **N. Vandencasteele**,* Universite Libre de Bruxelles, Belgium*, P. Viville,* Materia Nova, Belgium*, R. Lazzaroni, M. Hecq,* Université Mons Hainaut - Materia Nova, Belgium*, D.G. Castner,* University of Washington*, F. Reniers*, Universite Libre de Bruxelles, Belgium

The adsorption of bovine serum albumine on surfaces is usually a first good test for potential biological applications. In this study, PTFE surfaces were exposed to a remote RF oxygen plasma. The plasma was characterized using optical emission spectrometry, whereas the PTFE surface was characterized using monochromatized XPS, dynamic contact angle and atomic force microscopy. The modified surfaces are then exposed to BSA. The presence of protein was then evidenced by the presence of the N1s peak in the XPS spectrum, by AFM images, and by the change in the contact angle. We show that at low plasma power (or DC-bias) and short treatment times, the contact angle decreases, leading to slightly more hydrophilic surfaces. Small amounts of oxygen (up to 5%) are detected on the surface. BSA adsorbs on these surfaces. An increase in the plasma power leads to an increase of the sample roughness and to an increase of the hydrophobicity. On superhydrophobic (angles above 160°) surfaces, BSA does not absorb any more. No oxygen is present in the XPS spectrum. A correlation was established between the change of the contact angle, the amount of adsorbed protein and the roughness. It is shown that the decrease of the contact angle, and the hysterisis between the advancing and receding angles are good probes for protein adsorption.

5:00pm **PS2+BI-ThA10 Composition and Structure Study of the AP Plasma Deposited Hydrophobic Thin Film**, ***C. Chen***, *W. Hsieh, C. Liu, W. Hsu, C. Lin*, Industrial Technology Research Institute, Taiwan

In this study hydrophobic thin films were prepared by plasma enhanced chemical vapor deposition at atmospheric pressure by means of two layer compositions on the surface of glass. The bottoms were using Ar and hexamethyldisilazane (HMDSN) as the carrier and monomer gases respectively to deposit silicon oxide and offer microstructure. The deposited glasses were further coating a hydrophobic layer using fluoroalkylsilane (FAS) as the chemical precursor. Meanwhile, to evaluate the effects of fluorine contained of the water repellency of substrate, CF4 was introducing into the plasma zone during plasma depositions. The chemical structure of the thin film was characterized using X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopies (FTIR) measurement. Morphologies and topography of the coatings were examined by scanning micro spectroscopy (SEM) and atomic force microscopy (AFM). FTIR measurement indicated that a SiO2 layer can successful deposit on the glass and the porosity of the thin films was direct proportion with thin film thickness. The SEM results indicated that the thickness of the thin films increasing almost linearly with coating times and the thickness was about 143nm after three times of deposition. AFM results reveal nano-clusters were well distributed on the surface after two layers deposition but introducing CF4 during deposition will slightly reduced the roughness because of decompose reaction between CF4 plasma and the Si atom in the thin films. The deconvolution of the C1s core-level spectra and atomic ratio from XPS measurement indicate FAS can be react and deposited on the top layer. The contact angles of the double layers was 134.0 degree which are great than traditional fluoro-polymer such as polytetrafluoroethylene (PTFE). Otherwise, bypass introducing CF4 during plasma polymerization the contact angle will increased to 143.3 degree indicate the AP plasma can be used to deposit super hydrophobic thin film on the glass surface.

Biomaterial Interfaces

Room: 4C - Session BI-ThP

Biomaterial Interfaces Poster Session

BI-ThP1 HAp/Chitosan Composites from Electrospinning Technique, *F. Vazquez-Hernandez, S.A. Lopez-Haro,* UPIITA-IPN, Mexico, ***C.O. Mendoza-Barrera***, Universidad Veracruzana, Mexico, *M.A. Melendez-Lira,* CINVESTAV-IPN, Mexico, *M.L. Albor-Aguilera, E. Diaz-Valdez*, FCFM-IPN, Mexico

Human bone is a hidroxyapatite HAp ($HCa_5O_{13}P_3$) and collagen based composite. Actually many methods and techniques have been developed and applied to design advanced materials for bone replacent. An stratergy to biomimic bone tridimentional structure, composition and mechanical properties is mimic it at nanoscale. In other words, selectively incorporate nano particules of apatites into a polymeric matrix by controlling structure and composition of the fibres. In this work we present the preliminary results of HAp/Chitosan composites prepared via electrospinning technique. Chitosan fibers were spun from aqueous solutions (pH= 3) and nano particles of HAp were added prior to the deposition. Prior and after the deposition the compositional and structural characteristics were verified by using scanning electron microscopy SEM, energy dispersive spectroscopy EDS, x-ray diffraction XRD, Raman spectroscopy and Fourier transform infrared spectroscopy FTIR. Energy dispersive spectroscopy and x-ray diffraction confirmed that the mineral deposits were hydroxyapatite and calcium phosphate monobasic MCP ($CaH_4\ O_8\ P_2$), both of them apatites present in bone while Fourier transform infrared studies FTIR showed the characteristic 1220-1020 cm^{-1} chitosan region in agreement with Raman results.

BI-ThP2 TOF-SIMS Imaging Study on Water Soluble and Organic Soluble CdSe/ZnS Core/Shell Quantum Dots, ***T.G. Lee***, *H. Min,* KRISS, Korea, *Y. Kim, S.J. Lim,* POSTECH, Korea, *D.W. Moon,* KRISS, Korea, *S.K. Shin*, POSTECH, Republic of Korea

Water-soluble CdSe/ZnS core/shell quantum dots capped by 3-mercaptopropionic acid(MPA) have been studied by using time-of-flight secondary ion spectroscopy (TOF-SIMS) imaging analysis. TOF-SIMS images provide direct evidence of local chemical information on the quantum dot surfaces. The water-soluble quantum dots can be conjugated with protein, DNA and other biomolecules, and thus be useful to applying to bioimaging and biosensing. These quantum dots were generated by converting original organic soluble ligands to MPA ligands. We characterized both surfaces of water soluble and organic soluble quantum dots and confirmed the successful exchange of ligands by using TOF-SIMS images of ligand molecules and molecular metal adducts.

BI-ThP3 The Effect of TOF-SIMS Ion Sources on the Fragmentation Pattern of Adsorbed Protein Films, ***S. Muramoto,*** University of Washington, *D.J. Graham,* Asemblon, Inc., *R. Michel,* University of Washington, *M.S. Wagner,* Proctor & Gamble Co., *T.G. Lee, D.W. Moon,* Korea Research Institute of Standards and Science, *L.J. Gamble, D.G. Castner*, University of Washington

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is a powerful surface analysis technique for the characterization of organic surfaces due to its high surface sensitivity, molecular specificity, and high mass resolution. However, the fragmentation patterns of positive secondary ions from adsorbed proteins, produced by primary ion bombardment, are complex due to multiple fragments originating from each of the 20 amino acids present in proteins. Therefore, the multivariate analysis technique principal component analysis (PCA) was used to identify fragment peaks that vary significantly between spectra. This study utilized Cs^+, Au^+, Au_3^+, Bi^+, Bi_3^+, Bi_3^{++}, C_{60}^+, and C_{60}^{++} ion sources to generate mass spectra for five single-component proteins (albumin, fibrinogen, lysozyme, collagen and immunoglobulin G) adsorbed onto mica. With the use of PCA, we observed differences in fragmentation patterns among the ion sources for all proteins. However, the differences between ion sources for a given protein were smaller than the differences between different proteins. This allows the type of protein to be identified regardless of the ion source used. For each of the five proteins, the fragmentation patterns generated from Cs^+, Au^+ and Au_3^+ ions were differentiated at the 95% confidence level. For the Bi ion sources, the fragmentation patterns from the Bi^+ ions were differentiated from the fragmentation patterns from the Bi_3^+ and Bi_3^{++} ions at the 95% confidence level, but the fragmentation patterns from the Bi_3^+ and Bi_3^{++} ion sources could not be differentiated. From the PCA loadings it appears that there may be a dependency between the mass of the ion source and the number of carbon atoms in the emitted secondary ions (i.e., the Au ions may produce more smaller fragments than the Cs ions). Also, sampling depth may play a role in the observed differences between monoatomic and the cluster ion beams. The results from this study show how the combination of TOF-SIMS with PCA can be used to identify the influence of primary ion type on secondary ion fragmentation patterns.

BI-ThP4 Synthesis of CdSe Nanoparticles by Nanocaged Protein sHSP 16.5, ***J.-W. Park***, *S.H. Moh, N.H. Kim, K.K. Kim, Y.H. Roh*, Sungkyunkwan University, Korea

Synthesis of semiconductor nanoparticles is one of the interesting research fields in nanotechnology. The semiconductor nanoparticles are applicable to various electronic device, optical devices and bio sensors. Since the energy levels of the semiconductor nanoparticles are quantized depending on their size and shape, therefore it is important to synthesize semiconductor nanoprticles with the same size and shape. Quantum-effect devices were actively investigated to solve these problems. In this study, we performed biomimetic approach to control these factors by using inorganic material (CdSe nanoparticle) and organic nanocaged proteins. Protein cage architectures can be utilized as size- and shape-constrained reaction environments for nanomaterial synthesis. Biomimetic fabrication could be performed in the optimized conditions using small heat shock protein 16.5 (sHSP16.5) mutant and ion complex of cadmium and selenium. A directed nucleation could be achieved through the specific residues on the negatively charged center domain of sHSP16.5. CdSe nanoprticles had the excellent size uniformity in the core of the mineralized sHSP16.5. For 2D array on the Si wafer, we treated chemical such as 3-aminopropyltrethoxysilane (APTES). The mineralized sHSP16.5 has highly binding affinity on the APTES treated Si wafer, which the reason is hydrogen interactions between amine group of the APTES and carboxyl group of sHSP16.5. These results give us to control arrangement of quantum dots on the Si wafer. This biomimetic approach will be possible to achieve advanced floating gate memory devices and single electron transistor in the near future.

BI-ThP5 Visible Light Activated Conjugated Polyelectrolytes as Antimicrobials, ***T Corbitt***, *L.K. Ista, K. Ogawa, S. Chemburu, G.P. Lopez,* University of New Mexico, *K. Schanze,* University of Florida, *D. Whitten*, University of New Mexico

Conjugated polyelectrolytes (CPs) are polymers that contain ionic solubilizing groups. These materials are water soluble and feature strong visible absorption and fluorescence. Recently we have shown that CPs functionalized with cationic groups such as trimethyl ammonium (1) and diazobicyclooctane bis quaternary salt (2) polyphenyleneethynylene display efficient light-activated biocidal activity against a variety of microorganisms including Escherichia coli, Cobetia marina, Pseudomonas aeruginosa and Bacillus anthracis, Sterne spores. When bacteria are mixed with aqueous solutions of the polymers the CPs form a surface coating on the microorganism. Irradiation of the bacteria surface-coated with polymers 1 or 2 leads to efficient deactivation of the bacteria as revealed by culturing and live/dead assays. We have also demonstrated that CPs are effective against bacteria when coated at near monolayer coverage on a surface. An additional format currently under investigation involves polymers with the repeat unit of 1 grown covalently from the surface of silica nanoparticles and microspheres (SGCP). The polymer coatings of the SGCPs are more rough and appear from studies using confocal fluorescence microscopy and electron microscopy to protrude from the surface. We find that the SGCP entrap Cobetia marina; live-dead assays of entrapped bacteria kept in the dark and irradiated with visible light reveal that bacteria entrapped on the surface of a 5 micron SGCP are killed while those kept for a similar period in the dark remain viable. We are currently determining whether the light-activated pathogen killing requires molecular oxygen and, if so, whether singlet oxygen is a key intermediate. Our observation that bacteria are not only killed but also degraded suggests that singlet oxygen may subsequently generate much more powerful reactive oxygen species. Structure-property relationships are being developed to optimize the biocidal effect of specific CPs, with the ultimate objective being to develop a new class of polymer-based materials and coatings that are highly effective antimicrobial agents with broad-spectrum activity. The CPs offer advantages over low molecular weight compounds in that they are relatively stable and easily adapted to form robust coatings. Their ease in processing indicates they can be incorporated into filtration devices, foams, paints and fibers for diverse protection applications.

BI-ThP6 Determination of the Surface pK_d of Carboxylic Acid and Amine-Terminated Alkanethiols Using Surface Plasmon Resonance, *K.P. Fears, **S.E. Creager**, R.A. Latour*, Clemson University

Alkanethiol self-assembled monolayers (SAMs) are widely used as model surfaces because they form very well-characterized monolayers over a broad range of surface chemistries. An important property for SAMs with ionizable functional groups is the surface dissociation constant (pK_d), which defines the charge-density for a given pH solution. Using surface plasmon resonance (SPR) spectroscopy, we developed a method for the direct measurement of the pK_d of COOH and NH_2-SAMs by combining the ability of SPR to detect the changes in mass concentration close to a surface and the shift in ion concentration over the surface as a function of surface charged density. An analytical study was first performed to theoretically predict the general shape of the expected SPR plots by calculating the excess mass of salt ions over the SAMs as a function of the difference between the solution pH and surface pK_d. SPR studies were then conducted to measure the shift in salt concentration as a function of bulk pH, with the resulting data being used to determine the pK_d for HS-$(CH_2)_{11}$-COOH SAMs to be 7.4 ± 0.2 (N=4, mean ± 95% C.I.) and 6.5 ± 0.4 (N=4, mean ± 95% C.I.) for HS-$(CH_2)_{11}$-NH_2 SAMs . These methods present a way to calculate the pK_d for charged SAM surfaces that is non-disruptive and minimally interactive with the surface, thus providing an accurate and direct measure of the pK_d of the surface.

BI-ThP7 Effect of PNIPAAM Chain Length on Thermal Responsive Properties and Cellular Adhesion, ***B.P. Andrzejewski**, J. Fenton, L.K. Ista, G.P. Lopez*, University of New Mexico

Poly(N-isopropyl acrylamide) (PNIPAAm) is one of the most extensively studied responsive materials exhibiting a thermally triggered molecular transition effecting hydration. Our poster will present work on surface grafted PNIPAAm by the controllable reaction of atom transfer radical polymerization (ATRP). We use ATRP to investigate the effects of polymer length on both thermal responsive and biological adhesion properties. X-ray photoelectron spectroscopy (XPS), contact angle measurements, ellipsometric thicknesses and biological attachment studies will be used to probe thermal responsiveness to the polymer chain length. By precisely varying the polymer length, we give insight into the chemical and physical properties of the surface that govern its thermal responsiveness and resulting cellular adhesive properties.

BI-ThP8 Universal Route for Synthesis of Protein Resistant Polymer Brushes by Surface-Initiated Atom Transfer Radical Polymerization, ***A. Hucknall**, A.J. Simnick,* Duke University*, B.D. Ratner,* University of Washington*, A. Chilkoti*, Duke University

The ability to resist non-specific protein adsorption is an important enabling technology for the design of biosensors and biomedical implants. We have previously shown that surface-initiated atom transfer radical polymerization (SI-ATRP) of oligoethylene glycol methacrylate (OEGMA) can be used to create exceptionally robust and non-fouling surface coatings. In our previous studies, examples of substrates modified with poly(OEGMA) brushes were limited to materials which support the formation of self-assembled monolayers (SAMs) capable of initiating SI-ATRP, such as gold, silicon and metal oxides. However, the surfaces of many technologically relevant materials, such as plastics, do not support SAM formation. This paper presents a simple method to modify the surface of virtually any material with a robust, non-fouling poly(OEGMA) brush by SI-ATRP. Surface initiator layers capable of supporting SI-ATRP were formed by two routes: (1) plasma polymerization of 2-chloroethyl methacrylate and (2) dip-coating of poly(vinylbenzyl chloride). These layers were then used to initiate SI-ATRP of OEGMA. XPS revealed that the poly(OEGMA) brushes formed by either route were indistinguishable from those formed on alkanethiol SAMs on gold. The ability of the resulting poly(OEGMA) layers to resist non-specific protein adsorption was evaluated by incubating the surfaces in undiluted fetal bovine serum for 12 hours-subsequent XPS analysis showed no detectable protein adsorption. Substrates were also incubated for 12 hours in a solution of human umbilical vein endothelial cells in serum containing media and no cell attachment was observed on the poly(OEGMA) coated substrates.

BI-ThP9 Modeling Force versus Distance Profiles of Terminally Anchored Poly (N-isopropyl acrylamide) with Self-Consistent Field Theory, ***S. Mendez**, B. Andrzejeweski, D.H. Keller, H.E. Canavan, G.P. Lopez, J.G. Curro,* University of New Mexico*, J.D. McCoy*, New Mexico Tech

Tethered polymers are widely used to control surface properties such as wettability or cell adhesion. By making thin films out of polymers that are thermo-responsive, we can modulate surface properties with changes in temperature. Specifically, we use poly(N-isopropyl acrylamide) (PNIPAM) since this exhibits lower critical solution temperature (LCST) behavior near 32°C in water. At temperatures below the LCST, the polymer is hydrated and swollen, whereas above the LCST, the polymer collapses, and when tethered, the surface becomes more hydrophobic. In the past we reported on a method of synthesizing thin films of terminally anchored PNIPAM from self-assembled monoloyers using atom transfer radical polymerization.[1] We used neutron reflectivity techniques to measure the polymer brush structure at temperatures above and below the solution LCST. To model the temperature-induced structural changes of these brushes, we employed self-consistent field (SCF) theory using as input the Flory-Huggins chi parameter extracted from the experimental polymer solution phase diagram.[2] As a continuation of that work, we used the SCF theory to calculate the force between the PNIPAM brush and a test wall as a function of wall separation distance, i.e., we generated force-distance profiles. The parameters that we varied were temperature, polymer surface coverage and molecular weight, and the interaction between the PNIPAM and the test wall. AFM techniques were employed to obtain force-distance profiles of PNIPAM samples. We found that the force-distance profiles predicted by the theory were in qualitative agreement with those from experiment. Our ultimate goal is to employ theoretical predictions to guide future efforts to optimize tethered PNIPAM for cell attachment/detachment applications.

[1] Yim et al, Macromolecules 2006, 39, 3420.
[2] Mendez et al, Macromolecules 2005, 38, 174.

BI-ThP10 Electrochemical Behavior of Electroactive Species in Nucleic Acid Monolayers of Different Chain Length, ***K. Wang**,* Polytechnic University*, M.A. Gaspar,* Columbia University*, R.A. Zangmeister,* National Institute of Standards and Technology*, R. Levicky*, Polytechnic University

Monolayers of immobilized nucleic acids (DNA) are promising experimental models for investigating fundamental properties of charged polymers at solid-liquid interfaces. We have investigated the charging behavior of single-stranded DNA polyelectrolyte brushes. In this study, voltammetric behavior of hexaamineruthenium(III) chloride (RuHex) in end-tethered single-stranded DNA monolayers of different strand lengths is investigated. The surface coverage of non-labeled DNA chains was determined independently with X-ray photoelectron spectroscopy (XPS). Our results show that, for DNA chains varying from 5 to 100 thymine nucleotides, the reduction potential of $RuHex^{3+}$ counterions associated with the DNA monolayer is predominantly a function of chain surface coverage and is rather insensitive to the chain length. However, the total charge passed to reduce the counterions to the 2+ oxidation state is predominantly a function of the total nucleotide number, given by the product of chain surface coverage and chain degree of polymerization. A model is proposed to explain the observed behavior. The dynamic evolution of the reduction peak area and potential are also investigated, providing a picture of the time dependence of the adsorption of $RuHex^{3+}$ into the monolayers. The research provides a method to estimate the chain coverage of non-labeled, end-tethered DNA chains with various chain lengths.

BI-ThP11 Tuning the Zeta Potential of Poly-L-Lysine Substrates for the Selective Immobilization of Nanoparticles and Biomaterials, ***N. Farkas**, J.A. Dagata,* National Institute of Standards and Technology*, K.F. Pirollo, E.H. Chang*, Georgetown University Medical Center

Colloidal systems composed of nanoparticles must be charge stabilized in order to prevent aggregation. In many applications of nanotechnology it is necessary to immobilize nanoparticles intact and dispersed on a substrate so that high-resolution imaging and characterization can be carried out. An essential first step in sample preparation therefore involves appropriately matching the zeta potential of nanoparticles in solution to the zeta potential of the substrate surface and adjusting the pH and ionic strength of the solution environment. Here we report a method for preparing patterned substrates with regions of optimally tuned surface zeta potential by combining fluid scanning probe microscopy and a recently reported surface zeta potential apparatus [P. J. Sides et al., Langmuir 22 (2006) 9765]. Specifically, we vary the zeta potential of a poly-L-lysine substrate over a range of approximately $-60 \text{ mV} < \zeta < +100 \text{ mV}$ by exposure to UV/ozone and control nanoparticle adsorption from effectively zero to full monolayer coverage. Exposure through a mask produces local regions with positive and negative surface charge resulting in selective adsorption of nanoparticles. We demonstrate attachment, followed by particle size distribution, and zeta potential measurements, for hard and soft nanoparticles including 10- to 80-nm diameter gold nanoparticles and 30- to 80-nm diameter liposomes.

BI-ThP12 Chemical Characterization of Taq DNA Polymerase Adsorption on Different Surfaces, ***R. Canteri**, R. Dell'Anna, S. Forti, L. Lunelli, L. Pasquardini, L. Vanzetti, M. Anderle, C. Pederzolli*, Fondazione Bruno Kessler-irst - Italy

PCR (polymerase chain reaction) represents the most widely used method for amplification of defined DNA sequences in medical and biological applications. The most recent innovative technologies are based on PCR

reaction miniaturization. In fact, reductions in reagent consumption lower costs and increase scalability, enabling genome-wide approaches. Due to the increased surface-to-volume ratio of microchip PCR, a crucial role is played by the internal surface. Effects related to the non specific surface adsorption of PCR reagents (e.g. the replicating enzyme DNA polymerase) become significant and may reduce the efficiency of DNA amplification. In this study we investigate the Taq (Thermus aquaticus) DNA polymerase adsorption on different material surfaces, namely silicon (with different deposited oxide layer), pyrex glass, chromium nitride, cyclic olefin co-polymer (COC), polycarbonate (PC), poly(methyl methacrylate) (PMMA), and polydimethysiloxane (PDMS) surface. We carry out analyses via time of flight secondary ion mass spectrometry (ToF-SIMS), providing a physical-chemical surface picture, and via immunofluorescence by using anti-Taq DNA polymerase monoclonal antibody, giving the surface distribution and the amount of the protein. By combining these different techniques a deeper insight into the mechanisms governing the non specific surface adsorption of PCR reagents is possible.[1]

[1]This work was accomplished in the framework of LaTEMAR (Laboratorio di Tecnologie Elettrobiochimiche Miniaturizzate per l'Analisi e la Ricerca - Laboratory of Miniaturized Electrobiochemical Technologies for Analysis and Research), Centre of Excellence funded by MIUR (Italian Ministry for Education, University and Research) grants - FIRB 2003-2004 - for public/private structures involved in research fields characterized by strategic value.

BI-ThP13 Surface Chemical and Geometric Determination of Neuronal Migration on Patterned Surfaces, ***W. Wang***, *A. Natarajan, P. Molnar, S. Lambert, M. Das, M. Stancescu, N. Bhargava, J.J. Hickman*, University of Central Florida

Highly organized neuronal networks exist in the brain and are formed by appropriate neuron migration during the developmental stage. In vitro, engineering the appropriate neuron migration pathways and controlling the destination of single migrating neurons has been a challenge due to the insufficient understanding of integrated physiochemical mechanisms that regulate this process. In this work, we show that with controlled surface chemistry and proper design of pattern geometry of the substrate, single neuron migration pathways and destinations can be controlled. However, more importantly, the mechanism of this migration of how the neuron populations respond to the different surface chemistry and pattern geometry has been investigated by time lapse morphological analysis. We recorded the dynamic neuron migration that occurs during the formation of patterned two-neuron circuits using embryonic hippocampal neurons, where the somal adhesion sites, axon and dendrites outgrowth pathways are precisely determined. The cellular patterns were maintained in a defined serum free culture medium. Substrate surfaces were modified with self-assembled monolayers and patterns formed by laser ablation through a quartz photo mask. The surface chemistry was analyzed utilizing X-ray photoelectron spectroscopy and contact angle measurements. The patterns were visualized by metal deposition and optical profilometry. The neurons were characterized by static and dynamic morphological analysis and immunocytochemistry. Synaptic connections were determined by dual-patch clamp electrophysiology. The neurons were observed to migrate to designed somal adhesion sites by leading edge extension along the designed neurite pathways using a previously unknown process. After soma attachment, axon and dendrite outgrowth then continued along the designed pathways. This result will contribute to the methods of designing neuron network formation in culture, for the study of neuron migration in vitro and sensor design and fabrication.

BI-ThP14 Surface Characterisation and Biological Response of Enzymatically Tailored, Surface-Coupled Polysaccharides Pectic Hairy Regions*, ***G. Ceccone***, *D. Gilliland, I. Liakos, F. Rossi,* EC-JRC-IHCP, Italy*, M. Morra, C. Cassinelli, G. Cascardo,* Nobil-Bio-Ricerche, Italy, *C. Della Volpe,* University of Trento, Italy, *R. Verhoef, H. Schols*, University of Wageningen, The Netherlands

The exploitation of the bio-active properties of polysaccharides covalently linked to materials surfaces is a rapidly growing area of biomaterials surface science. Recent findings on bioactivity of plant carbohydrate polymers are spurring an activity of biomolecular scouting and suggest that pectic polysaccharides are promising flexible molecules for novel bioactive surfaces. In this work we have investigated the properties of surface linked pectic rhamnogalacturonans(RG-I) fractions(MHRs) obtained by commercial enzyme preparations of homogenized vegetable tissue. MHRs were covalently linked to different substrates, namely polystyrene(PS), Titanium(Ti) and polycarbonates(PC) surfaces aminated by glow discharge plasma and analysed by XPS, ToF-SIMS, AFM, and contact angle measurement. Cell adhesion experiments using L-929 fibroblasts and Aortic Smooth Muscle cells(SMC) were performed to evaluate the effect of the MHRs nature on cell adhesion. Moreover, cells growth and specific alkaline phosphatase (ALP) activity of osteoblast-like SaOS2 cells were also measured. Surface analyses of different samples indicate that coupling of MHRs polysaccharides was successful for all substrates. XPS analysis of plasma amminated PS shows significant amount of N (13at%) related to the presence of amino groups. After MHRs coupling, strong increase of O/C ratio is detected, whilst nitrogen signal is still present indicating that the thickness of MHR layer is below the XPS sampling depth(<10nm).ToF-SIMS analysis supports the XPS data: amminated surfaces present CxHyN peaks expected in allylamine-like films, whilst large fragments peaks (m/z>250amu) are observed both on parent and on surface-coupled polysaccharides.AFM force-separation curves show that immobilization of MHRs significantly affects the interfacial forces with the absence of any attractive interaction until repulsive contact is reached. Results of cells experiments reveal that the structure of the immobilized MHRs (long vs short hair) has great influence on adhesion, morphology and cells enzymatic activity. In particular the long-haired MHRs are found less adhesive. Interestingly, specific ALP activity of the modified surfaces is upregulated respect to that of the control, suggesting that MHRs coated surfaces present interfacial properties suitable for osteoblast differentiation.

*This work is carried out within the EU STREP Project # 517036 (PECTICOAT).

BI-ThP15 Optically Responsive Nano-Composite Layers for Quantitative Label-Free Detection of Biospecific Interactions, *P. Buecker, E. Trileva, M. Himmelhaus,* ***R. Dahint***, University of Heidelberg, Germany

In a recent paper,[1] we presented a novel way of preparing densely packed, metal coated nanoparticle films for the label-free detection of binding events. The layer system is composed of dielectric nanoparticles, which are adsorbed onto a plain gold surface and subsequently metallized by deposition of gold colloid prior to electroless plating. Upon reflection of white light, the layers exhibit pronounced extinction peaks which shift to higher wavelengths when molecules adsorb onto the surface. For the same concentration and incubation time of octadecanethiol, an about fivefold higher red-shift of the extinction maximum was observed than reported for conventional surface plasmon resonance (SPR).[2] However, as no quantitative information existed on the amount of adsorbed molecules, which may be different for our nanoparticle layers and the plain gold surfaces used in standard SPR, no clear decision could be made regarding their sensitivity towards adsorbate layer thickness or mass density. Thus, the goal of the present study was to accurately determine the mass sensitivity of the nano-composite films in order to facilitate quantitative studies of binding events. For this purpose, self-assembled monolayers of simple and ethylene glycol terminated alkanethiols with various chain lengths were prepared on the nanoparticle coated substrates. The measured red-shift of the extinction spectrum upon molecule adsorption was related to the thickness and mass density of the films as determined by X-ray photoelectron spectroscopy. Special attention was paid to the question whether sensitivity decreases with increasing film thickness, as this could limit the use of the nanoparticle layers for biosensing applications, which often involve the detection of high molecular weight molecules. Experiments on antigen/antibody interactions show that the sensitivity factors determined for thin organic films can also be used to quantify the amount of surface bound protein in immunoreactions.

[1] R. Dahint, E. Trileva, H. Acunman, U. Konrad, M. Zimmer, V. Stadler, M. Himmelhaus, Biosensors & Bioelectronics, in press.

[2] L. S. Jung, C. T. Campbell, J. Phys. Chem. B 2000, 104, 11168-11178.

BI-ThP16 Synthesis Biocompatible Gold Nanorods, *S. Reed,* ***B. Ayres***, Portland State University

The ability to tune the optical properties of metal nanoparticles by changing their size and shape make them an ideal and diverse tool for biomedical applications. Challenges remain to utilizing nanomaterials for in vivo medical applications. By selecting benign compounds as synthons for nanoparticles it is predicted that toxicity can be greatly reduced. Furthermore, the resulting synthetic waste can be minimized and the process made more environmentally friendly and safe. We report nanoparticle-liposome composite materials that are stable, water soluble, and anticipated to be benign. Specifically, soy lecithin has recently been used to synthesize particles with these characteristics. These lipids are a cheap, readily available and non-toxic ligand for the synthesis of gold particles. Soy lipids form liposomes that function as nanoreactors in which particles form. We aim to change the shape and size of particles by manipulating these nanoreactors allowing for tuning of their optical properties. The optical applications of gold nanoparticles are of particular interest. Design of particles with a particular shape and size are desirable for use in vivo. Rod shaped nanoparticles absorb near infrared light that penetrates into deep tissue and presents a unique possibility to locate and treat maladies non-invasively. Using soy lecithin as a ligand, particles of a uniform size distribution can be created with reproducible results. Soy components have also shown promise for shape control of particles. Using these naturally occurring ligands, a series of gold nanoparticles have been synthesized and characterized. The resulting nanoparticles are stable for long periods with little aggregation. UV-Visible spectroscopy and transmission electron microscopy have been used to characterize size and

shape of the resulting nanoparticles. It is believed a plethora of components contained in soy could also play a role in particle synthesis. We have isolated a small number of these compounds and identified them using NMR and mass spectrometry. Active components have been identified which contain linoleate tails. Ethyl linoleate has been positively identified and its ability to effect the shape of the gold nanoparticles is under study. Synthetic ethyl linoleate is being used in parallel with ethyl linoleate recovered from the soy lecithin to reveal its role in shape control of the nanoparticles as well as the rate of reaction in particle synthesis.

BI-ThP17 Surface Plasmon Resonance Microscopy Combined with a Novel Microfluidic System for High-Throughput Immunoassays, *J. Liu, M.A. Eddings, B.K. Gale,* ***J.S. Shumaker-Parry***, University of Utah

Surface plasmon resonance (SPR) microscopy provides quantitative, real-time information about adsorption and desorption on an SPR-active sensor surface with high spatial resolution. Label-free, high-throughput analysis of biomolecule interactions is made possible by combining patterned biomolecule immobilization with SPR microscopy. Typically, the biomolecules are immobilized ex situ using a pin-based microspotting device with many parameters that must be well-controlled in order to create an active and reliable sensor surface. We demonstrate the combination of a high-throughput microfluidic device with SPR microscopy for quantitative, in situ antibody immobilization. The microfluidic device provides 48 separate flow channels that can be used simultaneously for antibody immobilization and subsequent antibody-antigen interaction analysis. Because the biomolecules can be immobilized in situ, exposure to harsh environments can be avoided, a major benefit for protein immobilization. In addition, the biomolecule immobilization process can be monitored in real time by SPR microscopy and characterized quantitatively. Applications in immunoassay development for studying patient immunogenic response to antibody-based drugs will be described.

BI-ThP18 Characterization of Plasma Polymerized Immunosensor by XPS, SPR and ToF-SIMS, ***E.N. Newman***, *F. Cheng, L.J. Gamble, K. Bomsztyk, D.G. Castner*, University of Washington

Surface treatment of polypropylene with plasma polymerized acrylic acid (PPAA) has been used to fabricate an immunosensor. This study examines both the amount and the bioactivity of the immobilized antibody. A comparison of PPAA - based strategy to the traditional coupling chemistry onto self-assembled monolayers was done using X-ray photoelectron spectroscopy (XPS) and surface plasmon resonance (SPR). XPS results for the PPAA surfaces indicate that 1) PPAA can be deposited onto various substrates (e.g., polypropylene, Si wafer and gold), 2) ~ 50% of the low-power, deposited PPAA film dissolves after soaking overnight in water, and 3) the N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide / N-hydroxysuccinimide method activates carboxyl groups at PPAA surface and allows proteins to be coupled to it. SPR provides a quantitative comparison of the amount of anti-ferritin immobilized by two strategies and the amount of ferritin the immobilized anti-ferritin binds. In addition, time-of-flight secondary ion mass spectrometry (ToF-SIMS) is used to investigate the structure of the PPAA surface as well as the structure of the immobilized anti-ferritin. Our results suggest that the PPAA - based strategy improves the biological activity and stability of the immobilized antibodies.

BI-ThP19 Label-free Plasmonic Detection of Biomolecular Binding by a Single Gold Nanorod, ***G.J. Nusz***, *S.M. Marinakos, A.C. Curry,* Duke University, *A.B. Dahlin, F. Hook,* Lund University, Sweden, *A. Wax, A. Chilkoti*, Duke University

We report the use of isolated gold nanorods as plasmonic transducers to detect the binding of streptavidin to biotin-conjugated nanorods on a surface in real time by tracking the wavelength shift of their resonant scattering spectrum using a darkfield microspectroscopy system. The limit-of-detection of streptavidin binding by a single biotinylated nanorod is 1 nM. An analytical model is presented that provides a rational framework from which optimal nanoparticle geometries can be predetermined for a specified detection experiment. In addition, the model provides a method for quantifying the number of molecules bound to the nanorod surface based on the resonant wavelength shift. Signal saturation occurs upon the binding of approximately 700 streptavidin molecules to the surface of gold nanorods that are immobilized on a glass substrate, with a detection limit of approximately 40 protein molecules per nanorod. The limits of molecular detection that can be theoretically achieved by a single nanorod are discussed as well as the prospects of detection of single receptor-analyte binding events in real-time.

BI-ThP20 Preparation of High Resolution SPR Imaging Microarray Using Polymeric Micropatterns, *J. Jung,* Seoul National University, Korea, *J. Yuk, K. Ha,* Kangwon National University, Korea, ***J. Hyun***, Seoul National University, Korea

In this paper, we demonstrate a simple method to fabricate SPR imaging microarrays using polymer micropatterns. The use of thick polymeric micropatterns in imaging SPR microarray passivates the region by removing SPR signals completely or by saturating the SPR signal far beyond the detection range in SPR imaging. Two schemes to create polymeric micropatterns on the surface were demonstrated by micropatterning a thick insulating layer before depositing a metal layer or after depositing a metal layer. A biotin-streptavidin system was successfully performed to verify the systematic binding of biomolecules and the adsorption of cell culture media on the microarrays was quantitatively characterized. This SPR microarray can be applied in a variety of areas including protein adsorption, cell research, diagnosis of diseases, and more.

BI-ThP21 Development of Metal Polymer Based Hybrid Micro Channel Network in bio-MEMS, *M. Dhayal, R.R. Pandey, S.C. Jain,* ***K.K. Saini***, National Physics Laboratory, India

In this paper development of cost effective polymeric material based micro devices using soft lithography techniques had been discussed. This includes polymer based micro fluidic devices for bioengineering applications to study the self-assembly of bio-molecules in bio-MEMS. We had investigated the effects of diffusing transition metals into soft polymer based micro channel network (MCN) to control the surface charge and chemistry. These inorganic coatings and metal particle diffused into the MCN has been derivatised with various organic functionalities. This process can lead to novel characteristics of these devices for different bioengineering applications including bio-molecules separation and controlled electro osmotic mobility.

BI-ThP22 Fabrication of Micron-sized Retroreflectors, ***T. Sherlock***, *S.M. Kemper, P. Ruchhoeft,* University of Houston, *R.L. Atmar,* Baylor College of Medicine, *R.C. Willson*, University of Houston

We have fabricated micron-sized retroreflectors (structures that return incident light directly back to the source) and have shown that they are extremely bright and detectable over a large range of angles when inspected with a simple optical microscope. These retroreflectors are part of an ultra-sensitive detector platform for sensing small quantities of virus particles, bacteria, DNA, RNA, or any variety of molecules of interest. In particular, we are targeting our first generation sensor to detect Norwalk virus particles. In this proposed system, the base of the retroreflector is decorated with antibodies to the virus, and, if present, the virus particles are captured by this surface. After capture, gold nanoparticles, coated with a secondary antibody, are introduced into the system, attach to the virus, and drastically reduce the retroreflector brightness with a specific, well-understood spectral signature. If no virus is present, the reflectivity is unaffected. We have measured the reflectivity versus 40nm diameter gold nanoparticle surface density and have found a 40% reduction in signal for 100 nanoparticles per square micron when illuminated with broad-band light. The base of the retroreflector is about 4 square microns in size, yielding a detection sensitivity of hundreds of particles. Further optimization of particle size and illumination wavelength is expected to increase this sensitivity substantially. Retroreflectors are fabricated by coating a silicon wafer with 2.5 microns of polyimide and 200nm of resist. A lithography step is used to generate the retroreflector pattern as openings in the resist and a 50nm thick nickel coating is deposited using thermal evaporation. After a lift-off step, which leaves behind only the nickel that coated the base of the resist openings, the patterns are transferred into the polyimide in an O_2/CF_4 reactive ion etch, leaving the retroreflecting structure with very straight relatively smooth walls. Next, gold is evaporated to coat the base of the structure and a directional evaporation step is used to cover all but the sensor base with aluminum. The gold is selectively functionalized with amine-reactive thiol molecules which serve as a platform for attaching antibodies, oligonucleotides, or other detector molecules.

BI-ThP23 Patterning Live Bacterial Cells for Biological Applications, *Z.Y. Suo,* ***R. Avci***, *P. Rugheimer, X.H. Yang, Y. Idzerda, D.W. Pascual*, Montana State University

The immobilization of live bacterial cells in a controlled fashion in well-defined patterns will have many applications in biosensors, and in biomedical and fundamental biological studies. The surface antigens, fimbriae and flagella of Salmonella typhimurium and Escherichia coli and corresponding antibodies were used to demonstrate the immobilization of live bacteria in well-defined patterns. The leashing of live bacterial cells was achieved on antibody-modified substrates of gold, silicon and glass. The tendency of bacterial cells to remain adhered (leashed) only to the antibody-modified areas was used to fabricate microarray patterns whose

size can be controlled down to a micron scale. Patterns are generated with either a focused ion beam milling system or a microplotter. Cells patterned in this way retain their viability for at least six hours in a PBS buffer solution and are capable of regeneration if incubated in a growth medium. These microarray patterns can serve as prototype sensors which are able to capture targeted pathogens including bacteria, virus and proteins. For example, we have already demonstrated the use of such microarrays as a bacterial sorting system, in which a pre-targeted bacterial strain is captured and isolated from a mixed culture of microorganisms. The technique offers a reliable approach for fundamental microbiological research on the behavior of bacteria in an immobilized mode, as microorganisms respond to environ¬mental changes. For example, we observed that individual S. typhimurium cells gradually adjust their orientation from a "lying down" to a "standing up" position during regeneration, presumably trying to leave their position in search of more food. In such a struggle, immobilized cells produce a larger number of flagella as compared with planktonic cells, as confirmed by SEM and AFM studies.

BI-ThP24 A Bio-MEMS Device for Measuring Contractile Forces of Cultured Myotubes on Microfabricated Cantilevers*, **K.A. Wilson**, M. Das, P. Molnar,* University of Central Florida*, K.J. Wahl, R.J. Colton,* U.S. Naval Research Laboratory*, J.J. Hickman*, University of Central Florida

The boom in the semiconductor manufacturing industry of the past three decades has yielded a vast array of techniques for fabricating devices with micro to nano-scale features. Concomitantly, advances in biotechnology have opened new avenues for the application of these technologies in the form of gene and protein arrays, lab-on-a-chip devices and biological micro-electromechanical systems (Bio-MEMS). To date, application of these technologies has largely focused on the study of biomolecules and single cells or cell types. However, these technologies also hold great promise for the study of complex cellular and tissue interactions that are of critical importance when developing new drug therapies for disease and catastrophic injury. A tissue type of broad interest with regard to drug development and basic cell biology is skeletal muscle, which is affected by a variety of pathological conditions such as Parkinson's, ALS, and muscular dystrophy. For this reason we have developed a Bio-MEMS device based on microfabricated silicon cantilevers for the controlled, real-time interrogation of embryonic rat myotubes as a high-throughput test bed for drug discovery and basic science. The cantilevers were fabricated using standard photolithographic and micromachining techniques. The surfaces of the cantilevers were then modified using an amine-terminated alkylsilane SAM (DETA) to improve cellular adhesion, growth and differentiation. Dissociated embryonic rat myocytes were cultured for 7-10 days in a defined serum-free medium until contractile myotubes had formed. Monitoring and interrogation of the myotubes was accomplished using an AFM detection system of our own design, which consisted of a microscope, photodiode laser, position sensitive detector, field stimulation chamber, and a computer with data acquisition and analysis software. This simple system allows the real-time, high-throughput analysis of the physiological properties of the contracting myotubes. With this system we have shown the ability to selectively control the frequency and magnitude of myotube contraction as well as induce and observe physiological phenomena such as tetanus and fatigue. Contraction forces were calculated using a modified Stoney's equation for bending of a cantilever due to thin film stress. Ongoing work will allow the selective patterning and co-culture of neuronal cell types with myotubes for studying the neuromuscular junction and in vitro biological circuits.

BI-ThP25 Surface Modification and Photolithographic Patterning of Microelectrode Arrays for Cell-Based Biosensor Applications*, **A. Natarajan**, N. Bhargava, P. Molnar, M. Das, J.J. Hickman*, University of Central Florida

A major research area in the field of cell-based biosensors and pharmaceutical testing is the development of functional cell-based networks and their integration with silicon-based platforms. The development of a hybrid cell-electrode system could also aid in understanding neuronal circuits, cardiac physiology and function, and the interactions between these cells. Using surface chemistry, we have developed a technique to first modify the surface of commercially available microelectrode arrays and glass using self-assembled monolayers (SAM). This is done using a cell-adhesive SAM like trimethoxysilylpropyldiethylene-triamine (DETA). Patterns are then made on the microelectrode arrays using a photolithography based method with a quartz mask that defines and guides neuron attachment and development. The patterned surface is then backfilled with an appropriate cell-repulsive SAM like perfluoroalkyltrichlorosilane (13F). The surfaces have been characterized by both X-ray Photoelectron Spectroscopy (XPS) and contact angle measurements. Dissociated Embryonic hippocampal cells, in a serum-free medium, were cultured on these patterned microelectrode arrays in order to create neuronal networks with directed synaptic connectivity. The cells were characterized by morphological analysis as well as immunocytochemistry. The functionality of these networks was further studied using long term recording of the electrical activity of these cells in the presence and absence of toxins. We will report on the characterization of these devices as well as the methods developed for toxin detection and elucidation using these devices. We have also developed a technique to look at myocardial tissue function by manipulating surface chemistry in order to pattern and guide the growth of actively beating monolayers of neonatal rat cardiomyocytes on glass. These devices have also been characterized for their response to toxins and its effect on cardiac physiology. These hybrid systems are being used to further study basic neuronal networks and cardiac physiology properties like functional reentry. More importantly the devices are being applied to study toxic effects in pharmacological evaluation and to study disease models like Arrhythmia.

Biomaterial Interfaces

Room: 609 - Session BI-FrM

Microbioanalytical Systems

Moderator: T. Boland, Clemson University

8:00am **BI-FrM1 Tools and Platforms for Single-Cell Biology**, ***B.A. Parviz***, University of Washington **INVITED**

Genome sequence data enable global, high throughput approaches that link genomic differences to the physiological outcomes that ultimately lead to disease. However, the Achilles heel of global approaches is reliance on averaged cell populations. It is becoming increasingly clear that cells are highly heterogeneous in both gene expression and phenotype. Cellular heterogeneity confounds the interpretation of the link between genomics, phenotype, and disease and also the interpretation of response to therapeutic intervention. In fact, heterogeneity underlies most failures of current therapies for cancer. In order to realize the promise of genomics in curing major diseases, it will be necessary to elucidate pathways involved in disease at the single-cell level, to both understand and manipulate the inherent heterogeneity. The goal of the Microscale Life Sciences Center (MLSC) is to develop cutting edge technology for multi-parameter analysis of single cells, and apply this technology to the understanding of biological questions characterized by cellular heterogeneity. The current focus is on disease pathways, and the vision is to address pathways to disease states directly at the individual cell level, at increasing levels of complexity that progressively move to an in vivo understanding of disease. This presentation provides an overview of the activities in the Center and efforts undertaken to this date to develop automated platforms for trapping and manipulation of single cells, micro-scale and nano-scale photonic methods to measure cells parameters such as oxygen consumption, single-cell protein analysis, and nano-scale electronic methods to monitor extracellular molecular traffic

8:40am **BI-FrM3 Reversible Biofunctionalization and Catalytic Activity of a Metabolic Pathway Enzyme in Reusable BioMEMS Devices**, ***X.L. Luo***, *A.T. Lewandowski, G.F. Payne, R. Ghodssi, W.E. Bentley, G.W. Rubloff*, University of Maryland

We report a reversible biofunctionalization strategy for assembling a biocatalytically-active enzyme at localized sites in reusable bioMEMS devices as a first step toward an experimental platform for metabolic engineering applications, e.g. drug discovery. We prefabricate an integrated bioMEMS device and exploit a non-permanent sealing and package design which supports programmable bio-component assembly at selected sites in the completely packaged microfluidic environment. The aminopolysaccharide chitosan is utilized as the interfacial biofunctionalization material for (1) the chemical signal-guided conjugation of chitosan to a Pfs enzyme through its pro-tag, which is genetically engineered at the C-terminal of Pfs and is activated by tyrosinase for the conjugation, and (2) the electric signal-guided electrodeposition of the Pfs-chitosan conjugate to a selective electrode under negative bias in the microfluidic channel. With biofunctionalizaton complete, the Pfs-mediated enzymatic reaction is performed by introducing the substrate S-adenosylhomocysteine (SAH) into the microchannel for conversion into the products S-ribosylhomocysteine (SRH) and adenine. Reaction solutions are collected and analyzed with high-performance-liquid-chromatography (HPLC). Initial studies show that high conversions are achieved at low flow rates, while much lower conversions are achieved at high flow. After reaction, a mild acid wash removes the assembled Pfs-chitosan conjugate and thus removes the catalytic activity. Subsequent biofunctionalization with Pfs-chitosan re-establishes enzymatic activity comparable to the original, illustrating the reversibility of the enzyme assembly and the reusability of our bioMEMS. Storage in PBS buffer at room temperature for 4 days degraded the conversion efficiency only slightly, demonstrating robustness of the assembled enzyme. This work demonstrates (a) the reversible assembly of a Pfs enzyme at a specific electrode address and (b) the efficacy of the metabolic pathway enzyme Pfs in the bioMEMS: Pfs converts SAH to SRH and adenine in one step of a multi-step cell-signaling process (autoinducer-2 production), a quorum sensing phenomenon that determines pathogenicity of bacteria. These results illustrate the capability of the bioMEMS as an efficient and reusable platform in screening potential enzyme inhibitors as antimicrobial drug candidates. This work is supported in part by the Robert W. Deutsch Foundation.

9:00am **BI-FrM4 SPR Microscopy and its Applications to High-Throughput Analyses of Biomolecular Binding Events and their Kinetics**, ***C.T. Campbell***, University of Washington, *G. Kim*, Lumera Corp.

Surface plasmon resonance (SPR) sensing has long been used to study biomolecular binding events and their kinetics in a label-free way. This approach has been extended to SPR microscopy more recently, which is an ideal tool for probing large microarrays of biomolcules for their binding interactions with various partners and the kinetics of such binding. SPR microscopes now make it possible to simultaneously monitor binding kinetics on >1300 spots within a protein microarray with a detection limit of below 1 ng per cm2, or <100 femtograms per spot (< 2 million protein molecules) with a time resolution of 1 s, and spot-to-spot reproducibility within a few percent. The method is label free and uses orders of magnitude less of the precious biomolecules than standard SPR sensing. It also gives the absolute bound amount and binding stoichiometry. Experiments designed to demonstrate that this approach is capable of high-throughput kinetic studies of the binding of small (200-500 Da) ligands onto large protein microarrays will be described.

9:20am **BI-FrM5 Studies of Electroosmotic Mobilities, and Protein Adsorption in Plasma Polymerised Microchannel Surfaces**, *M. Salim, B.J. O'Sullivan, G.J.S. Fowler, G. Mishra, P.C. Wright*, ***S.L. McArthur***, University of Sheffield, UK

Miniaturisation has found its application in many biological, medical and pharmaceutical devices. Its advantages over macroscale systems include fast analysis time and lower sample consumption. These microfluidic devices use of electroosmotic flow (EOF) as the mean of fluid transport, although variations and inconsistencies in EOF may interfere with device performances. Plasma polymerisation can be used to deposit thin films of various chemical functionalities and properties. This study investigates the electroosmotic behaviour and stability of a range of different plasma polymerised and polymer grafted surfaces before and after contact with protein solutions. The results illustrate that plasma polymerised surfaces exhibit high stabilities, enabling EOF runs of more than 3 days without deterioration. Critically, EOF measurments of surfaces after contact with protein solutions illustrate significant changes in EOF with very low levels of protein adsorption. While this is detrimental to the function of the device, it does suggest that these measurements may be a sensitive probe for in channel biofouling.

9:40am **BI-FrM6 Attomolar Toxin Detection with Semi-Homogeneous Assays**, ***S.P. Mulvaney***, *K.M. Myers, P.E. Sheehan, L.J. Whitman*, Naval Research Laboratory

Assays for biomolecular detection are ideally both multiplexed and sensitive, metrics that often require conflicting solutions. For example, microarrays use spatial location for multiplexing, but target capture on the surface will ultimately be diffusion-limited. Alternatively, homogeneous assays offer very efficient target capture, but typically require multiple label types to multiplex. In our semi-homogeneous immunoassays we use microbeads for both target capture and labeling to leverage the advantages of both approaches. The sample is first mixed with secondary antibodies and microbeads that are functionalized against the secondary host. Target molecules are thereby captured onto the beads via the secondary antibodies. The target-loaded beads are then captured onto an antibody microarray, and controlled fluidic forces are applied to preferentially remove nonspecifically bound beads.[1] Finally, the remaining beads are counted to determine the target(s) concentration. Utilizing such assays, we have achieved multiplexed toxin detection, including aM detection of SEB, in <20 min in a variety of complex matrices. Micrometer-scale sensors and beads are optimal for detecting nanoscale biomolecules with practical sensitivity and speed.[2] However, the blending of micro- and nano-scales in such assays leads to some interesting relationships. In diffusion-limited, solid-phase assays, it takes hours-to-days for fM targets to accumulate on a nanosensor, but only seconds-to-minutes on a microsensor. In addition, microscale labels enable fluidic forces to be applied to achieve greater sensitivity and fewer false positives than possible with nanoscale labels. Finally, in contrast to nanoscale labels, individual microbeads can be easily detected. We believe the size mismatch between label and target contributes to the extreme sensitivity of our assays. Each microbead-label confines a very small volume beneath the contact area, thereby creating a high local concentration of target molecules and capture/label antibodies. This confinement greatly increases the effective binding constant, suppressing dissociation and detachment of the label. The relatively large bead size also contributes to the unusual log-linear dose response curves we obtain, that span up to nine orders of magnitude.

[1] Mulvaney, et al., Biosens. Bioelectron., in press.

[2] Sheehan and Whitman, Nano Lett. 5, 803 (2005).

10:00am **BI-FrM7 Affinity Capillary Electrophoresis and Other Separations on a Microfluidic Format**, ***F.A. Gomez**, A. Brown, M. Piyasena, A. Gaspar, S. Stevens*, California State University, Los Angeles **INVITED**

In this paper, we describe the design and development of novel microfluidic devices (MDs) for electrophoretic and chromatographic separations. One study details our work on through-a-chip partial filling affinity capillary electrophoresis (PFACE) to estimate binding constants of ligands to receptors using as model systems carbonic anhydrase B (CAB, EC 4.2.1.1) and vancomycin from Streptomyces orientalis. Using multilayer soft lithography (MSL), a MD consisting of fluid and control channels is fabricated from poly(dimethylsiloxane) (PDMS) and fitted with an external capillary column. Multiple flow channels allow for manipulation of a zone of ligand and sample containing receptor and non-interacting standards into the MD and subsequently into the capillary column. Upon electrophoresis the sample components migrate into the zone of ligand where equilibrium is established. Changes in migration time of the receptor are used in the analysis to obtain a value for the binding interaction. In a second study we describe the development and study of a disposable and inexpensive MD, fabricated from PDMS incorporating conventional chromatographic reversed-phase silica particles (C18) without the use of frits, permanent physical barriers, tapers or restrictors. A novel external in-line magnetic valve allows for facile packing of the particles. Clamping- and anchor-effects providing the stability and the compactness of the packing were observed. A fiber optics assembly is incorporated onto the chip for detection of species. Food dyes and cephalosporin antibiotics were used to demonstrate the chromatographic applicability of this chip-based chromatographic packing.

10:40am **BI-FrM9 Dual Magnetic-/Temperature-Responsive Nanoparticles for Microfluidic Separations and Assays**, ***J.I. Lai**, J.M. Hoffman, M. Ebara, A.S. Hoffman, P. Stayton*, University of Washington

Magnetic nanoparticle (mNP) technologies have attracted attention for diagnostic applications because mNPs display potential advantages in their diffusive and superparamagnetic properties. However, their small particle size reduces the magnetic capture efficiency. Therefore, there is a need to design mNPs that can be effectively separated without compromising their diffusive properties. Here we have developed an approach that addresses this challenge in the microfluidic channel setting by using mNPs synthesized from temperature-responsive polymeric micelles. Telechelic poly(N-isopropylacrylamide) (PNIPAAm) polymer chains were synthesized with a dodecyl tail at one end and a reactive carboxylate at the opposite end by the reversible addition fragmentation transfer technique. These PNIPAAm chains self-associate into nanoscale micelles that were used as dimensional confinements to synthesize the mNPs. The Mössbauer spectrum of the resulting mNPs shows two broad quadrupolar doublets with chemical shifts of 0.38 and 0.21 mm/s suggesting that the mNPs contain only Fe^{3+}. The X-ray diffraction spectrum confirms the mNP is γ-Fe_2O_3. The mNPs exhibit a layer of carboxylate-terminated PNIPAAm chains as a corona on the surface. The carboxylate group was used to functionalize the mNPs with biotin which was subsequently bound to streptavidin. The biotinylation increases the mNP size from 7 to 11 nm. The functionalized mNPs can be reversibly aggregated in solution as the temperature is cycled through the PNIPAAm lower critical solution temperature (LCST). The LCST of the mNP is ~ 32 ^oC before and after the biotinylation. While the magnetophoretic mobility of the individual mNPs below the LCST is negligible, the aggregates formed above the LCST are large enough to respond to an applied magnetic field. The mNPs can associate with biotinylated targets as individual particles, and then subsequent application of a combined temperature increase and magnetic field can be used to magnetically separate the aggregated particles onto the poly(ethylene glycol)-modified polydimethylsiloxane channel walls of a microfluidic device. When the magnetic field is turned off and the temperature is reversed, the captured aggregates re-disperse into the channel flow stream. The dual magnetic- and temperature-responsive nanoparticles can thus be used as soluble reagents to capture diagnostic targets at a specific channel position with temporal control.

11:00am **BI-FrM10 Rapid Analysis of Species Separation in Multianalyte Integrated Micro/Nano Fluidic Chips using Multivariate Image Analysis**, ***K. Artyushkova**, M. Bore, A. Evangelista-Lara, G.P. Lopez*, University of New Mexico

This study investigates the potential of multivariate methods (MVA) for identifying electrokinetic separation and estimating velocities of moving species based on analysis of imaging datasets from microfluidic and nanofluidic devices. We have developed an image analysis methodology based on MVA of temporal datasets that is capable of identifying velocities of at least two molecular species from the images where no visible separation of the species has occurred. Among multivariate analysis methods examined are Principal Component Analysis (PCA), Multivariate Curve Resolution (MCR), PARAFAC (parallel factor analysis) and Independent Component Analysis (ICA). These methods allow one to fully exploit the data by analyzing all pixels within images and using the temporal dimension, in contrast with manual methods of visual inspection of images or traditional image processing methods. The methodology has been developed and tested temporal images acquired by fluorescence microscopy capturing separation within nanochannels, microchannels and gel electrophoresis of charged dyes and model protein receptor/ligand systems.

11:20am **BI-FrM11 High Throughput Pharmacological Screening using Cell-Based Biosensors**, ***K. Varghese***, Medical University of South Carolina, University of Central Florida, *P. Molnar, N. Bhargava, M. Das,* University of Central Florida, *M.S. Kindy,* Medical University of South Carolina, *J.J. Hickman*, University of Central Florida, Medical University of South Carolina

In drug development there is a large demand for a system capable of high throughput screening, as well as stable long-term recordings. Cell-based biosensors (CBBs) have the potential to address this demand. CBBs work on the principle of a direct interface between electronics and biological cells, such that the electronics make it possible to quantify a change in the cells' immediate environment. The cell-electronics interface can be modified using different physical properties to vary the adhesive properties of cells. It then becomes possible to promote or inhibit cell adhesion, as well as support preferential attachment of one cell type over another. Cell-based electrophysiology can be broadly divided into two categories - 1) those based on intracellular potentials (e.g. use glass microelectrodes, as in patch clamping) and 2) those based on extracellular potentials. Our research focuses on the latter, wherein extracellular microelectrode arrays are used as a noninvasive and long-term approach for the measurement of biopotentials. The objective of this study is to develop a high throughput CBB where the cell-electronics interface is represented by neurons on Metal Microelectrode Arrays (MEAs). The sensor thus developed should be able to detect acute and chronic effects for a broad range of compounds, at a broad range of concentrations, on neuronal physiology. The inherent properties of this CBB also make it possible to obtain long-term recordings from the neurons. The CBB discussed here consists of a layer of cultured embryonic rat neurons on surface-modified MEAs. The interfaces were modified utilizing self-assembled monolayers and characterized utilizing XPS and contact angle measurements. This system was then used to study the time-dependant effects of Amyloid beta (a causative factor of Alzheimer's Disease) on embryonic rat neurons. Since long-term recordings were relatively easy to obtain, it was possible to observe the effects of amyloid beta, at nanomolar concentrations, over a period of ten hours or more, without cell death. A quantitative description of the effect of this compound on the neuronal system utilizing extracellular recordings will be described. The cells were also characterized by morphology as well as immunocytochemical analysis. Intracellular electrophysiological controls were also performed and will be compared to the results obtained with the solid-state devices.

Authors Index

Bold page numbers indicate the presenter

— A —

Abbott, N.L.: BI-TuP3, 13
Abu-Lail, N.I.: AS+BI+NS-TuM5, **5**
Alam, S.M.: BI-ThM6, 27
Albor-Aguilera, M.L.: BI-ThP1, 34
Alexander, M.R.: AS+BI+NS-TuM4, **5**; BI-TuA5, 11; BI-TuP14, 15
Allred, D.B.: BI-TuP4, 13
Almquist, B.D.: BI-WeM3, 19
Amrania, H.: AS+BI+NS-TuM9, 6
Amro, N.A.: NS1+BI-WeA1, **25**
Anderle, M.: BI-ThP12, 35
Anderson, D.G.: AS+BI+NS-TuM4, 5; BI-TuA5, 11; BI-TuP14, 15
Andrzejeweski, B.: BI-ThP9, 35
Andrzejewski, B.P.: BI-ThP7, **35**; MB+BI-MoM10, 1
Anglin, T.C.: BI+AS+NS-ThA3, **30**
Aoki, T.: AS+BI+NS-TuM13, 6
Apolinário, M.: MB+BI-TuP3, **18**
Apte, J.S.: BI+AS+NS-ThA7, **30**
Arlinghaus, H.F.: BI+AS+NS-ThA6, 30
Artyushkova, K.: BI-FrM10, **40**; MB+BI-MoM10, 1
Asplund, M.C.: BI-TuP13, 15
Atmar, R.L.: BI-ThP22, 37
Avci, R.: BI-ThP23, **37**; BI-TuP24, 17
Axelsson, P.: BI-ThM3, 27
Ayres, B.: BI-ThP16, **36**

— B —

Baker, C.C.: TR2+BI+NS+MN-ThM9, 29
Barron, A.E.: BI-WeA8, **24**
Beebe, Jr., T.P.: BI-WeM12, **20**
Bentley, W.E.: BI-FrM3, 39
Bergen, J.M.: BI-WeM9, 19
Berger, R.: BI-TuP1, 13
Bergström, G.: MB+BI-TuP2, 18
Bernards, M.T.: BI-TuM12, **8**; BI-TuP20, 16
Besenbacher, F.: BI-TuM11, 8
Bhargava, N.: BI-FrM11, 40; BI-ThP13, 36; BI-ThP25, 38; BI-TuM13, 8
Biswas, N.: BI-TuP21, **16**
Biswas, P.: BI-TuM5, 7
Blomfield, C.: BI+AS+NS-ThA9, 31
Boggs, M.E.: BI-WeM12, 20
Boland, T.: BI-TuA1, **11**
Bomsztyk, K.: BI-ThP18, 37
Bonander, J.: TR2+BI+NS+MN-ThM6, 29
Bonnet, N.: BI-TuM6, **8**
Bore, M.: BI-FrM10, 40
Bottenus, D.: NS1+BI-WeA5, 25
Boxer, S.G.: BI+AS+NS-ThA1, **30**
Bratescu, M.A.: BI-TuP4, **13**
Braun, P.V.: AS+BI+NS-WeA4, **22**
Brennan, A.B.: MB+BI-MoA8, **3**
Bretagnol, F.: NS1+BI-WeA10, 26
Briand, E.: BI-ThM3, 27
Brizzolara, R.A.: BI-TuP23, **17**; MB+BI-TuP1, 17
Brooks, B.R.: BI-TuM5, 7; BI-TuP11, 14
Brown, A.: BI-FrM7, 40
Brown, M.: BI-ThM9, 28
Browne, W.K.: BI-TuM2, 7
Broze, B.: PS2+BI-ThA9, 32
Brueck, S.R.J.: NS1+BI-WeA5, 25
Brueninghoff, S.: BI-TuP1, 13
Brukman, M.: TR2+BI+NS+MN-ThM9, 29
Buckhout-White, S.L.: NS1+BI-WeA4, **25**
Buecker, P.: BI-ThP15, 36
Burnham, N.A.: TR2+BI+NS+MN-ThM8, **29**
Burns, C.T.: BI-TuP8, 14
Burns, S.A.: BI-TuP7, **14**
Burnside, S.: BI-ThM9, 28

— C —

Callow, J.A.: MB+BI-MoM1, **1**
Callow, M.E.: MB+BI-MoM1, 1
Campbell, C.T.: BI-FrM4, **39**
Canavan, H.E.: BI+AS+NS-ThA8, **30**; BI-ThP9, 35; BI-TuP22, 16
Canteri, R.: BI-ThP12, **35**
Cascardo, G.: BI-ThP14, 36
Cassinelli, C.: BI-ThP14, 36
Castner, D.G.: AS+BI+NS-TuM3, 5; BI+AS+NS-ThA7, 30; BI-ThP18, 37; BI-ThP3, 34; BI-WeA10, 24; BI-WeM13, 20; PS2+BI-ThA1, 31; PS2+BI-ThA9, 32
Castro, L.: BI-TuP9, 14
Ceccone, G.: BI-ThP14, **36**; NS1+BI-WeA10, 26
Celina, M.C.: AS+BI+NS-WeA10, 23
Chan, J.: AS+BI+NS-WeA2, 22
Chang, E.H.: BI-ThP11, 35
Charitat, T.: NT+BI-WeM3, **20**
Chemburu, S.: BI-ThP5, 34; BI-TuP18, **16**
Chen, C.: PS2+BI-ThA10, **33**
Chen, C.P.: BI-WeM4, 19
Chen, K.-H.: BI-WeM4, 19
Chen, L.-C.: BI-WeM4, **19**
Chen, S.: BI-TuP20, 16; PL+BI-TuM9, 9
Chen, Z.: MB+BI-MoM8, **1**
Cheng, F.: BI-ThP18, 37; BI-WeM13, **20**
Chilkoti, A.: BI-ThP19, 37; BI-ThP8, 35; BI-WeA1, **23**; PL+BI-TuM9, 9
Choi, S.Y.: BI-TuP8, 14
Chong, K.S.L.: BI-WeM6, 19
Chow, D.: BI-WeA1, 23
Chu, P.: PL+BI-TuM3, 9
Coe, J.V.: PL+BI-TuM11, **10**
Cohen, S.R.: BI-TuM1, **7**
Cole, M.A.: BI-TuP10, **14**
Coles, J.M.: TR2+BI+NS+MN-ThM5, 28
Collier, G.: BI-TuP11, **14**
Colpo, P.: NS1+BI-WeA10, **26**
Colton, R.J.: BI-ThP24, 38
Conboy, J.C.: BI+AS+NS-ThA3, 30; BI-TuP21, 16
Corbitt, T: BI-ThP5, **34**
Corn, R.M.: PL+BI-TuM1, **9**
Coyne, J.A.: BI-WeA8, 24
Craighead, H.G.: TR2+BI+NS+MN-ThM1, 28
Cramer, H.-G.: AS+BI+NS-TuM12, 6
Creager, S.E.: BI-ThP6, **35**
Cui, X.: BI-TuA1, 11
Curro, J.G.: BI-ThP9, 35
Curry, A.C.: BI-ThP19, 37

— D —

Dabney, K.W.: BI-WeM12, 20
Dagata, J.A.: BI-ThP11, 35
Dahint, R.: BI-ThP15, **36**
Dahlin, A.B.: BI-ThP19, 37
D'Ambruoso, G.: BI-ThM9, 28
Das, M.: BI-FrM11, 40; BI-ThP13, 36; BI-ThP24, 38; BI-ThP25, 38
Davies, M.C.: AS+BI+NS-TuM4, 5; BI-TuA5, **11**; BI-TuP14, 15
Davis, T.N.: AS+BI+NS-TuM3, 5
Della Volpe, C.: BI-ThP14, 36
Dell'Anna, R.: BI-ThP12, 35
Desikan, R.: NS1+BI-WeA8, **25**; TR2+BI+NS+MN-ThM2, 28
Dhayal, M.: BI-ThP21, 37
Diaz-Valdez, E.: BI-ThP1, 34
Dickinson, G.H.: MB+BI-MoM11, 2
Ding, Y.-X.: BI-TuP17, **15**
Dong, M.: BI-TuM11, 8
Du, C.-X.: MB+BI-TuP2, 18
Ducker, R.E.: BI-WeM6, 19
Dukkipati, V.R.: NS1+BI-WeA9, **25**

— E —

Ebara, M.: BI-FrM9, 40
Eddings, M.A.: BI-ThP17, 37
Ederth, T.: MB+BI-MoM3, 1; MB+BI-TuP2, 18
Edvarsson, M.: BI-ThM3, 27
Edwards, J.S.: BI-WeA2, **23**
Efimenko, K.: MB+BI-MoA1, 3
Ekblad, T.: MB+BI-TuP2, **18**
Ekstrand, H.: BI-ThM3, 27
Elbert, D.L.: BI-TuM3, **7**
El-Desawy, M.: BI-WeM5, 19
Evangelista-Lara, A.: BI-FrM10, 40

— F —

Faria, S.: AS+BI+NS-WeA2, 22
Farkas, N.: BI-ThP11, **35**
Fears, K.P.: BI-ThP6, 35; BI-TuP16, **15**
Fenton, J.: BI-ThP7, 35
Fiorilli, S.: PS2+BI-ThA7, 32
Firestone, M.A.: BI-TuP8, **14**
Foerch, R.: BI-TuP1, **13**
Forti, S.: BI-ThP12, 35
Fowler, G.J.S.: BI-FrM5, 39
Fragala, J.: NS1+BI-WeA1, 25
Fredlake, C.P.: BI-WeA8, 24
Frenkel, J.: BI-TuM1, 7
Frogley, M.: AS+BI+NS-TuM9, 6
Fuhrer, M.S.: NS1+BI-WeA4, 25
Fujita, S.: BI-TuP27, **17**
Futrell, J.H.: AS+BI+NS-TuM6, 5

— G —

Gale, B.K.: BI-ThP17, 37
Gallagher-Gonzales, K.: BI+AS+NS-ThA8, 30
Gamble, L.J.: BI+AS+NS-ThA7, 30; BI-ThP18, 37; BI-ThP3, 34; BI-WeA10, 24; BI-WeM13, 20
Ganguly, A.: BI-WeM4, 19
Gao, X.: BI-WeM1, **19**
García, A.J.: BI-TuA10, 11
Gardella Jr., J.A.: BI-TuP7, 14
Gardella, J.A.: AS+BI+NS-TuM10, 6
Gaspar, A.: BI-FrM7, 40
Gaspar, M.A.: BI-ThP10, 35
Gates, R.: BI-TuP13, 15
Genzer, J.: MB+BI-MoA1, **3**
Ghodssi, R.: BI-FrM3, 39
Gilliland, D.: BI-ThP14, 36
Gölzhäuser, A.: BI-WeM5, 19
Gomez, F.A.: BI-FrM7, **40**
Gong, P.: BI-WeA4, **23**
Goo, W.: BI-ThM6, 27
Graham, D.J.: BI-ThP3, 34
Grainger, D.W.: BI-WeA10, 24
Grandin, H.M.: BI-ThM5, 27
Greenland, K.: AS+BI+NS-TuM3, 5
Grehl, T.: AS+BI+NS-TuM12, 6

Griesser, H.J.: BI-TuP10, 14
Grigoropoulos, C.P.: BI-WeM11, 20
Großmann, H.: BI-WeM5, 19
Guilak, F.: TR2+BI+NS+MN-ThM5, 28
Gupta, S.K.: NS1+BI-WeA4, 25
Guyer, D.R.: BI-TuM2, 7

— H —

Ha, K.: BI-ThP20, 37
Hadjar, O.: AS+BI+NS-TuM6, **5**; AS+BI+NS-WeA8, 22; PS2+BI-ThA2, 31
Hahn, C.: BI-TuM2, 7
Hähner, G.: BI-TuM6, 8
Hall, J.E.: NT+BI-WeM1, 20
Hammond, J.S.: AS+BI+NS-WeA1, 22
Han, S.M.: NS1+BI-WeA5, 25
Harbers, G.M.: BI-WeA10, 24
Hauch, K.D.: BI-TuA2, **11**
Havercroft, N.: AS+BI+NS-TuM12, 6
He, Y.: BI-TuP15, 15; BI-TuP20, **16**
Healy, K.E.: BI-WeM11, 20
Hecq, M.: PS2+BI-ThA9, 32
Heer, J.M.: PL+BI-TuM11, 10
Heinecke, J.W.: BI-TuM9, **8**
Heinrich, F.: NT+BI-WeM1, 20
Hemminger, J.C.: PL+BI-TuM3, 9
Hickman, J.J.: BI-FrM11, 40; BI-ThP13, 36; BI-ThP24, 38; BI-ThP25, 38; BI-TuM13, 8
Himmelhaus, M.: BI-ThP15, 36
Himpsel, F.J.: BI-TuP3, 13
Hirsh-Kuchma, M.: BI-TuM13, 8
Hlady, V.: BI-TuP17, 15
Hoffman, A.S.: BI-FrM9, 40
Hoffman, J.M.: BI-FrM9, 40
Holinga, G.: BI-TuM2, 7
Holm, E.R.: MB+BI-TuP1, **17**
Hone, J.C.: BI-ThM4, 27
Hook, F.: BI-ThP19, 37
Horbett, T.A.: AS+BI+NS-TuM3, 5; PS2+BI-ThA1, 31
Hovgaard, M.B.: BI-TuM11, **8**
Hower, J.C.: BI-TuP15, **15**; BI-TuP20, 16
Hristova, K.: BI-ThM1, **27**
Hsieh, W.: PS2+BI-ThA10, 33
Hsu, C.W.: BI-WeM4, 19
Hsu, W.: PS2+BI-ThA10, 33
Hsu, Y.-K.: BI-WeM4, 19
Huang, H.W.: NT+BI-WeM5, **21**
Hucknall, A.: BI-ThP8, **35**
Hunter, C.N.: BI-TuP19, 16
Hwang, D.H.: BI-WeM11, 20
Hyun, J.: BI-ThP20, **37**; BI-TuP26, 17

— I —

Ichiki, K.: AS+BI+NS-TuM13, 6
Idzerda, Y.: BI-ThP23, 37
Ishizaki, T.: BI-TuP27, 17; BI-TuP6, 13
Ista, L.K.: BI-ThP5, 34; BI-ThP7, 35; MB+BI-MoM10, **1**
Ito, E.: BI-TuA11, 12
Ivory, C.F.: NS1+BI-WeA5, 25

— J —

Jain, S.C.: BI-ThP21, 37
Janusz, S.: BI-TuP19, 16
Jay, G.D.: TR2+BI+NS+MN-ThM5, 28
Jiang, G.: AS+BI+NS-TuM3, **5**
Jiang, S.: BI-TuM12, 8; BI-TuP15, 15; BI-TuP20, 16; MB+BI-MoA10, **3**
Johnson, P.A.: BI-TuP2, **13**
Jones-Meehan, J.: BI-TuP23, 17
Jürgensen, A.: BI-TuP3, 13
Jung, J.: BI-ThP20, 37; BI-TuP26, 17

— K —

Kam, L.: BI-ThM4, 27
Kang, C.J.: BI+AS+NS-ThA4, 30
Karchin, A.: BI-TuP25, **17**
Kasemo, B.: BI-ThM3, 27; PL+BI-TuM4, 9
Keenan, M.R.: AS+BI+NS-WeA10, 23
Keller, D.H.: BI-ThP9, 35
Kellerman, L.: BI-TuP24, 17
Kemper, S.M.: BI-ThP22, 37
Kim, B.I.: TR2+BI+NS+MN-ThM6, **29**
Kim, G.: BI-FrM4, 39
Kim, H.D.: BI+AS+NS-ThA4, 30
Kim, K.K.: BI-ThP4, 34
Kim, N.H.: BI-ThP4, 34
Kim, O.: BI-TuP26, 17
Kim, Y.: BI-ThP2, 34
Kim, Y.J.: BI+AS+NS-ThA4, **30**
Kim, Y.S.: BI+AS+NS-ThA4, 30
Kindy, M.S.: BI-FrM11, 40
Kingshott, P.: BI+AS+NS-ThA9, **31**
Klimov, M.: BI-TuM13, 8
Knoll, W.: BI-TuP1, 13
Kollmer, F.: AS+BI+NS-TuM12, 6
Kootala, S.: BI-TuM13, 8
Kreuk, P.V.: BI-TuA1, 11
Krim, J.: TR2+BI+NS+MN-ThM9, 29
Kunze, A.: BI-ThM3, **27**
Kwon, E.: BI-WeM9, 19

— L —

LaBean, T.H.: PL+BI-TuM10, 10
Laflamme, M.A.: BI-TuA2, 11
Lai, J.I.: BI-FrM9, **40**
Lam, Y.: BI-ThM6, **27**
Lambert, S.: BI-ThP13, 36
Langer, R.: AS+BI+NS-TuM4, 5; BI-TuA5, 11; BI-TuP14, 15
Langhammer, C.: PL+BI-TuM4, 9
Larsson, E.: PL+BI-TuM4, 9
Laskin, J.: AS+BI+NS-TuM6, 5; AS+BI+NS-WeA8, 22; PS2+BI-ThA2, **31**
Latour, R.A.: BI-ThP6, 35; BI-TuM5, **7**; BI-TuP11, 14; BI-TuP16, 15
Lazarides, A.A.: PL+BI-TuM10, 10; PL+BI-TuM9, **9**
Lazzaroni, R.: PS2+BI-ThA9, 32
Lecuyer, S.: NT+BI-WeM3, 20
Lee, C.-Y.: BI-WeA10, 24
Lee, H.J.: PL+BI-TuM1, 9
Lee, J.: BI-TuP26, 17
Lee, J.Y.: AS+BI+NS-TuM1, 5
Lee, K.H.: BI+AS+NS-ThA4, 30
Lee, T.G.: AS+BI+NS-TuM1, 5; BI-ThP2, **34**; BI-ThP3, 34; BI-TuP26, 17
Leggett, G.J.: BI-TuP19, 16; BI-WeM6, **19**
Leung, M.C.: BI-TuA4, **11**
Levicky, R.: BI-ThP10, 35; BI-WeA4, 23
Lewandowski, A.T.: BI-FrM3, 39
Li, H.: BI+AS+NS-ThA3, 30
Liakos, I.: BI-ThP14, 36
Liedberg, B.: MB+BI-MoM3, **1**; MB+BI-TuP2, 18
Lim, S.J.: BI-ThP2, 34
Lin, C.: PS2+BI-ThA10, 33
Lin, J.S.: BI-WeA8, 24
Linford, M.R.: BI-TuP13, 15
Lippitz, A.: AS+BI+NS-WeA11, 23
Lisboa, P.: NS1+BI-WeA10, 26
Liu, C.: PS2+BI-ThA10, 33
Liu, D.L.: TR2+BI+NS+MN-ThM8, 29
Liu, J.: BI-ThP17, 37
Liu, X.: BI-TuP3, **13**
Lo Bartolo, S.: PS2+BI-ThA7, **32**
Lopez, G.P.: BI-FrM10, 40; BI-ThP5, 34; BI-ThP7, 35; BI-ThP9, 35; BI-TuP18, 16; MB+BI-MoM10, 1; NS1+BI-WeA5, 25
Lopez-Haro, S.A.: BI-ThP1, 34
Lösche, M.: NT+BI-WeM1, **20**
Luna-Morris, S.: BI-ThM5, 27
Lunelli, L.: BI-ThP12, 35
Luo, W.: PL+BI-TuM3, **9**
Luo, X.L.: BI-FrM3, **39**

— M —

Mannelli, I.: NS1+BI-WeA10, 26
Mantero, G.: PS2+BI-ThA7, 32
Marinakos, S.M.: BI-ThP19, 37
Martin, J.: TR2+BI+NS+MN-ThM8, 29
Martinez-Servantez, T.G.: BI-TuP2, 13
Matsuno, M.: BI-TuP6, **13**
Matsuo, J.: AS+BI+NS-TuM13, **6**
Mayorga, L.: AS+BI+NS-TuM3, 5; PS2+BI-ThA1, **31**
Mbugua, S.K.: BI-WeM12, 20
McArthur, S.L.: BI-FrM5, **39**; PS2+BI-ThA8, 32
McCoy, J.D.: BI-ThP9, 35
McCrea, K.R.: BI-TuM2, 7
McGillivray, D.J.: NT+BI-WeM1, 20
McLachlan, J.: AS+BI+NS-WeA2, 22
Meagher, R.J.: BI-WeA8, 24
Melendez-Lira, M.A.: BI-ThP1, 34
Melosh, N.A.: BI-WeM3, **19**
Mendez, S.: BI-ThP9, **35**
Mendoza-Barrera, C.O.: BI-ThP1, **34**
Meyer III, H.M.: NS1+BI-WeA8, 25; TR2+BI+NS+MN-ThM2, **28**
Meyer, R.L.: BI+AS+NS-ThA9, 31
Michel, R.: AS+BI+NS-TuM3, 5; BI-ThP3, 34; PS2+BI-ThA1, 31
Miller, B.P.: TR2+BI+NS+MN-ThM9, **29**
Miller, M.M.: PL+BI-TuM9, 9
Mills, D.L.: PL+BI-TuM3, 9
Min, H.: BI-ThP2, 34
Minowa, H.: AS+BI+NS-WeA3, 22
Mishra, G.: BI-FrM5, 39; PS2+BI-ThA8, **32**
Moeller, J.: BI+AS+NS-ThA6, 30
Moellers, R.: AS+BI+NS-TuM12, 6
Moh, S.H.: BI-ThP4, 34
Molnar, P.: BI-FrM11, 40; BI-ThP13, 36; BI-ThP24, 38; BI-ThP25, 38; BI-TuM13, 8
Montague, M.: BI-WeM6, 19
Moon, D.W.: AS+BI+NS-TuM1, **5**; BI-ThP2, 34; BI-ThP3, 34
Morra, M.: BI-ThP14, 36
Mortisen, D.J.: BI-TuA2, 11
Moulder, J.: AS+BI+NS-WeA1, 22
Mulvaney, S.P.: BI-FrM6, **39**
Murakoshi, K.: PL+BI-TuM13, **10**
Muramoto, S.: BI-ThP3, **34**
Murry, C.E.: BI-TuA2, 11
Myers, K.M.: BI-FrM6, 39

— N —

Na, K.: BI-TuP26, **17**
Nakata, Y.: AS+BI+NS-TuM13, 6
Natarajan, A.: BI-ThP13, 36; BI-ThP25, **38**
Nelson, M.: NS1+BI-WeA1, 25
Nemanich, R.J.: TR2+BI+NS+MN-ThM9, 29
Newman, E.N.: BI-ThP18, **37**
Nguyen, P.-C.: BI-WeA10, **24**
Niehuis, E.: AS+BI+NS-TuM12, **6**
Nihonyanagi, S.: AS+BI+NS-WeA3, 22
Ninomiya, S.: AS+BI+NS-TuM13, 6
Noguchi, H.: AS+BI+NS-WeA3, 22
Norton, P.R.: AS+BI+NS-WeA2, **22**
Nusz, G.J.: BI-ThP19, **37**
Nygren, P.: MB+BI-MoM3, 1

— O —

Ochsner, M.: BI-ThM5, 27
Ogawa, K.: BI-ThP5, 34; BI-TuP18, 16
Oh, Y.-J.: NS1+BI-WeA5, **25**
O'Hagan, D.: BI-TuM6, 8
Ohlhausen, J.A.: AS+BI+NS-WeA10, **23**
Olsen, J.: BI-TuP19, 16
Oncins, G.: TR2+BI+NS+MN-ThM7, **29**
Opdahl, A.: BI-WeA11, 24; BI-WeA5, **24**
Östblom, M.: MB+BI-MoM3, 1
O'Sullivan, B.J.: BI-FrM5, 39
Otzen, D.E.: BI-TuM11, 8
Özçam, A.E.: MB+BI-MoA1, 3

— P —

Pachuta, S.J.: AS+BI+NS-WeA9, **23**
Pandey, R.R.: BI-ThP21, 37
Pang, S.W.: NS1+BI-WeA9, 25
Parikh, A.: BI-ThM7, **28**
Park, B.-J.: AS+BI+NS-TuM5, 5
Park, I.K.: BI-WeM9, 19
Park, J.W.: BI-TuP26, 17
Park, J.-W.: BI-ThP4, **34**
Parker, K.K.: BI-TuA8, **11**
Parviz, B.A.: BI-FrM1, **39**
Pascual, D.W.: BI-ThP23, 37; BI-TuP24, 17
Pasquardini, L.: BI-ThP12, 35
Patrito, N.: AS+BI+NS-WeA2, 22
Payne, G.F.: BI-FrM3, 39
Pederzolli, C.: BI-ThP12, 35
Penner, R.M.: PL+BI-TuM3, 9
Perez-Dieste, V.: BI-TuP3, 13
Perez-Luna, V.H.: PL+BI-TuM5, **9**
Perrone, D.: PS2+BI-ThA7, 32
Petronis, S.: BI-ThM3, 27
Petrovykh, D.Y.: BI-TuP3, 13; BI-WeA11, **24**; BI-WeA5, 24
Petsev, D.N.: MB+BI-MoM10, 1; NS1+BI-WeA5, 25
Phelps, E.A.: BI-TuA10, **11**
Phillips, J.: AS+BI+NS-TuM9, **6**
Pillai, S.: BI+AS+NS-ThA9, 31
Pirollo, K.F.: BI-ThP11, 35
Pirri, C.F.: PS2+BI-ThA7, **32**
Piyasena, M.: BI-FrM7, 40
Plumridge, J.: AS+BI+NS-TuM9, 6
Pun, S.H.: BI-WeM9, **19**

— Q —

Quaglio, M.: PS2+BI-ThA7, 32

— R —

Racco, A.: MB+BI-TuP3, 18
Rading, D.: AS+BI+NS-TuM12, 6
Ramalingam, M.: BI-TuM13, **8**
Raman, S.: AS+BI+NS-WeA1, **22**
Ramsay, D.: MB+BI-MoM11, 2
Ramstrom, O.: BI-TuP12, **15**
Rangarajan, S.: BI+AS+NS-ThA6, 30
Ratner, B.D.: BI-ThP8, 35; BI-TuA2, 11
Reed, J.A.: BI+AS+NS-ThA8, 30; BI-TuP22, **16**
Reed, S.: BI-ThP16, 36
Reniers, F.: PS2+BI-ThA9, 32
Renner, T.: NS1+BI-WeA1, 25
Responte, D.J.: AS+BI+NS-TuM3, 5
Reynolds, N.: BI-TuP19, **16**
Rheinstadter, M.C.: NT+BI-WeM9, **21**
Ricciardi, C.: PS2+BI-ThA7, 32
Rittschof, D.: MB+BI-MoM11, 2
Rivolo, P.: PS2+BI-ThA7, **32**
Roberts, A.: BI+AS+NS-ThA9, 31
Rodriguez, K.R.: PL+BI-TuM11, 10
Roh, Y.H.: BI-ThP4, 34
Romero, E.: BI-TuP22, 16
Rosenblum, G.: BI-TuM1, 7
Rosenhahn, A.: MB+BI-MoA6, **3**
Rossi, F.: BI-ThP14, 36; NS1+BI-WeA10, 26
Rozhok, S.: NS1+BI-WeA1, 25
Rubloff, G.W.: BI-FrM3, 39; NS1+BI-WeA4, 25
Ruchhoeft, P.: BI-ThP22, 37
Rugheimer, P.: BI-ThP23, 37
Russell, Jr., J.N.: MB+BI-MoM11, 2

— S —

Saavedra, S.: BI-ThM9, 28
Sagi, I.: BI-TuM1, 7
Saini, G.: BI-TuP13, **15**
Saini, K.K.: BI-ThP21, **37**
Saito, N.: BI-TuP27, 17; BI-TuP4, 13; BI-TuP5, **13**; BI-TuP6, 13
Salim, M.: BI-FrM5, 39
Sanada, N.: AS+BI+NS-WeA1, 22
Sanders, J.E.: BI-TuP25, 17
Sanz, F.: TR2+BI+NS+MN-ThM7, 29
Sarikaya, M.: BI-TuP4, 13
Sarkar, S.: BI-TuP9, **14**
Sato, Y.: BI-TuP5, 13
Schanze, K.: BI-ThP5, 34; BI-TuP18, 16
Schauer, J.-C.: PS2+BI-ThA6, **32**
Schmidt, R.C.: BI-WeM11, **20**
Schnietz, M.: BI-WeM5, 19
Schols, H.: BI-ThP14, 36
Seantier, B.: BI-ThM3, 27
Sebba, D.S.: PL+BI-TuM10, **10**
Seki, T.: AS+BI+NS-TuM13, 6
Sheehan, P.E.: BI-FrM6, 39
Shepard, K.L.: BI-WeA4, 23
Sherlock, T.: BI-ThP22, **37**
Shin, S.K.: BI-ThP2, 34
Shudy, D.F.: BI-WeA5, 24
Shumaker-Parry, J.S.: BI-ThP17, **37**
Simnick, A.J.: BI-ThP8, 35
Simomura, M.: BI-TuA11, 12
Singh, G.: BI+AS+NS-ThA9, 31
Sinner, E.-K.: BI-TuP1, 13
Skinner, C.: BI-TuP2, 13
Slack, N.: BI-TuM1, 7
Smith, M.: BI-ThM5, 27
Sokolov, Y.: NT+BI-WeM1, 20
Solak, H.H.: BI-WeM5, 19
Somorjai, G.A.: BI-TuM2, 7
Spencer, N.D.: TR2+BI+NS+MN-ThM3, **28**
Stancescu, M.: BI-ThP13, 36; BI-TuM13, 8
Stayton, P.: BI-FrM9, 40
Stevens, S.: BI-FrM7, 40
Strobel, M.A.: AS+BI+NS-WeA9, 23
Stuart, S.J.: BI-TuM5, 7; BI-TuP11, 14
Subramaniam, V.: BI-ThM9, 28
Subramanian, A.: BI-TuP9, 14
Sullivan, S.P.: BI-WeM12, 20
Sun, E.: BI-ThM4, 27
Sunami, H.: BI-TuA11, **12**
Suo, Z.Y.: BI-ThP23, 37; BI-TuP24, **17**
Suzuki, M.: AS+BI+NS-WeA1, 22
Svedhem, S.: BI-ThM3, 27
Swaraj, S.: AS+BI+NS-WeA11, 23

— T —

Tadayyon, S.: AS+BI+NS-WeA2, 22
Takai, O.: BI-TuP27, 17; BI-TuP4, 13; BI-TuP5, 13; BI-TuP6, 13
Tampé, R.: BI-WeM5, 19
Tanaka, M.: BI-TuA11, 12
Taylor, M.: AS+BI+NS-TuM4, 5; BI-TuA5, 11; BI-TuP14, **15**
Teeters-Kennedy, S.: PL+BI-TuM11, 10
Textor, M.: BI-ThM5, **27**
Theilacker, W.M.: BI-WeM12, 20
Thissen, H.: BI-TuP10, 14
Thompson, D.W.: BI-TuP9, 14
Thundat, T.G.: NS1+BI-WeA8, 25; TR2+BI+NS+MN-ThM2, 28
Tian, H.: PL+BI-TuM11, 10
Tinazli, A.: BI-WeM5, 19
Torrent-Burgues, J.: TR2+BI+NS+MN-ThM7, 29
Trileva, E.: BI-ThP15, 36
Tsai, J.: BI-ThM4, **27**
Turchanin, A.: BI-WeM5, **19**
Twiss, J.L.: BI-WeM12, 20
Tyler, B.J.: BI+AS+NS-ThA6, **30**; BI-WeA10, 24

— U —

Unger, W.E.S.: AS+BI+NS-WeA11, **23**
Uosaki, K.: AS+BI+NS-WeA3, **22**
Urquhart, A.J.: AS+BI+NS-TuM4, 5; BI-TuA5, 11; BI-TuP14, 15

— V —

Valincius, G.: NT+BI-WeM1, 20
Vallini, I.: PS2+BI-ThA7, 32
Valsesia, A.: NS1+BI-WeA10, 26
Vandencasteele, N.: PS2+BI-ThA9, **32**
Vanzetti, L.: BI-ThP12, 35
Varghese, K.: BI-FrM11, **40**
Vazquez-Hernandez, F.: BI-ThP1, 34
Verhoef, R.: BI-ThP14, 36
Viville, P.: PS2+BI-ThA9, 32
Voelcker, N.H.: BI-TuP10, 14
Vogel, V.: BI-ThM5, 27; NS1+BI-WeA2, **25**

— W —

Waggoner, P.S.: TR2+BI+NS+MN-ThM1, **28**
Wagner, M.S.: BI-ThP3, 34
Wahl, K.J.: BI-ThP24, 38; MB+BI-MoM11, **2**; TR2+BI+NS+MN-ThM9, 29
Walker, G.: MB+BI-MoM5, **1**
Wandinger-Ness, A.: BI-TuP22, 16
Wang, C.H.: BI-WeM4, 19
Wang, K.: BI-ThP10, **35**
Wang, P.: AS+BI+NS-WeA8, **22**; PS2+BI-ThA2, 31
Wang, W.: BI-ThP13, **36**
Ward, R.S.: BI-TuM2, 7
Wark, A.W.: PL+BI-TuM1, 9
Wax, A.: BI-ThP19, 37
Weik, M.: NT+BI-WeM11, **21**
Wells, D.: AS+BI+NS-TuM10, **6**
White, R.G.: TR2+BI+NS+MN-ThM2, 28
Whitman, L.J.: BI-FrM6, 39; BI-WeA11, 24; BI-WeA5, 24
Whitten, D.: BI-ThP5, 34; BI-TuP18, 16
Wikström, A.: BI-ThM3, 27
Willis, D.E.: BI-WeM12, 20
Willson, R.C.: BI-ThP22, 37
Wilson, K.A.: BI-ThP24, **38**
Winter, J.: PS2+BI-ThA6, 32
Wooley, K.L.: MB+BI-MoA3, **3**
Woollam, J.A.: BI-TuP9, 14
Wright, B.: BI-TuP17, 15
Wright, P.C.: BI-FrM5, 39
Wu, Y.: BI-TuP18, 16
Wynne, J.H.: BI-TuP23, 17

— Y —

Yamamoto, R.: AS+BI+NS-WeA3, 22
Yamamoto, S.: BI-TuA11, 12
Yamashita, I.: PS2+BI-ThA3, **31**
Yan, M.: BI-TuP12, 15
Yang, X.H.: BI-ThP23, 37; BI-TuP24, 17
Yang, Z.: PS2+BI-ThA2, 31
Yegen, E.: AS+BI+NS-WeA11, 23
York, R.L.: BI-TuM2, **7**

Yuk, J.: BI-ThP20, 37

— Z —

Zaech, M.: BI-ThM3, 27
Zangmeister, R.A.: BI-ThP10, 35
Zauscher, S.: BI-ThM6, 27; BI-WeA1, 23; TR2+BI+NS+MN-ThM5, **28**
Zhang, F.: BI-TuP13, 15
Zhang, H.: BI-ThM9, **28**; BI-TuP2, 13
Zhang, M.: BI-TuA4, 11
Zheng, F.: BI-TuP3, 13
Zhensheng, L.: BI-TuA4, 11
Zhou, Y.: MB+BI-MoM3, 1
Zhu, Z.: AS+BI+NS-TuM11, **6**
Zorić, I.: PL+BI-TuM4, **9**