Monday Morning, October 2, 2000

Biomaterial Interfaces

Room 202 - Session BI+SS-MoM

Biological Surface Science

Moderator: D.G. Castner, University of Washington

8:20am **BI+SS-MoM1 Suspended Lipid Bilayers on Porous Alumina Surfaces**, ***C. Steinem***, *J. Drexler, C. Hennesthal,* WWU Muenster, Germany

The study presents a new class of artificial membrane system "suspended bilayers" closing up the gap between solid supported membranes (SSMs) and black lipid membranes (BLMs). Suspended bilayers were prepared on the basis of porous alumina surfaces which were produced by an anodic etch of neat alumina foils. Gold was evaporated on the upper surface of the porous material. The porous material was characterized by scanning electron microscopy (SEM), scanning force microscopy (SFM) and impedance spectroscopy. As revealed by SEM and SFM pores exhibit a mean diameter of 65 nm. Functionalization of the upper gold surface was achieved by self assembly of 3-mercaptopropionic acid (MPA) rendering the surface negatively charged at pH 8.6. To obtain suspended solid supported we fused unilamellar vesicles of N,N-dimethyl-N,N-dioctadecyl ammonium bromide varying in their sizes on the MPA-covered surface. Bilayer patches spanning the pores were visualized by scanning force microscopy in buffer using contact mode. The bilayer covered nanopores are thought to serve as second aqueous compartments of femtoliter volume providing enough space to incorporate transmembrane proteins and to generate ion gradients across the membrane together with the ability to use sensitive surface analysis tools.

8:40am **BI+SS-MoM2 Formation and Characterization of Stabilized Supported Phospholipid Bilayers**, ***S. Saavedra***, *E. Ross, J. Conboy, S. Liu, D.F. O'Brien,* University of Arizona

The utility of phospholipid bilayers as non-fouling coatings in molecular device technologies is hampered by the chemical and mechanical instability of these structures relative to (for example) alkylsiloxane self-assembled monolayers. Towards the use of lipid bilayers in applications such as biosensing, we have investigated two-dimensional polymerization as a strategy to stabilize planar supported lipid bilayers. UV-induced and redox-initiated polymerization have been used to prepare air-stable bilayers from phosphatidylcholine monomers containing sorbyl moieties in the acyl chains. Preparation of these structures using Langmuir-Blodgett-Kuhn and vesicle fusion techniques, and characterization of their physical and chemical properties, including nonspecific protein adsorption behavior, will be described.

9:00am **BI+SS-MoM3 Regulating Molecular Recognition and Self-assembly via Mechanical Forces: The Cell Adhesion Protein Fibronectin at Phospholipid Interfaces**, *A. Krammer, G. Baneyx, D. Craig,* University of Washington*; K. Schulten,* University of Illinois at Urbana-Champaign*;* ***V. Vogel***, University of Washington **INVITED**

While major progress has been made in the past to reveal how chemical factors regulate biorecognition, insight into pathways by which nature utilizes external forces to regulate biorecognition and signaling holds the potential for major new discoveries in biomedicine. Knowledge in this field is rudimentary since high-resolution crystallographic structures of biomolecules have mainly been obtained from equilibrated states. The role played by mechanical forces applied to the terminal ends of domains in regulating exposure of their recognition sites will be discussed here for the multidomain protein fibronectin. One of fibronectins many functions is to promote cell adhesion to surfaces. Starting from the equilibrium structure of fibronectin type III domains (FnIII), steered molecular dynamics simulations were applied to study the pathway by which their tertiary structures unravel under external forces. First we found that the accessibility of the cell recognition site on the FnIII10 domain, i.e. the RGD-loop, to integrins is reduced in an early stage of the forced unfolding pathway. Furthermore, forced unfolding studies of various fibronectin type III modules have shown that FnIII-7, FnIII-8, FnIII-9 and FnIII-10 differ considerably in their mechanical stability, and the simulations predict that FnIII-10 unfolds first. Finally, we have experimentally analyzed the pathway on which fibronectin assembles into fibrillar networks underneath phospholipid monolayers, and find again that mechanical forces are crucial to initiate its spontaneous self-assembly. Thus, spontaneous assembly of fibronectin into fibrils cannot be induced by adsorption to solid surfaces, yet it is the fibrillar state that allows cells to apply the forces needed to partially unfold fibronectin's domains.

9:40am **BI+SS-MoM5 New Methods for Patterning Fluid Lipid Bilayer Membranes on Solid Supports**, ***J.S. Hovis***, *S.G. Boxer,* Stanford University

Two new methods are introduced for patterning fluid lipid bilayer membranes on solid supports. These methods, called blotting and stamping, rely on the observation that supported lipid bilayers exhibit self-limiting lateral expansion. The consequence is that it is possible to pattern these fluid surfaces without modifying the underlying substrate. Together these methods constitute a simple and powerful approach for preparing patterned fluid lipid bilayers in nearly any geometry. One important application of these methods is the ability to create composition arrays of lipids and membrane associated proteins. These arrays allow the opportunity to study lipid-lipid, lipid-protein, and/or protein-protein interactions in a parallel fashion. Information gained about these important biological interactions will be highlighted.

10:00am **BI+SS-MoM6 The Interaction of Phospholipid Vesicles with Binary Alkanethiol/Hydroxythiol Monolayers**, ***V. Silin***, National Institute of Standards and Technology*; H. Wieder,* Max Planck Institute for Polymer Research, Germany*; J. Woodward, A. Plant,* National Institute of Standards and Technology

Surfaces modified by self-assembly have applications in sensors, diagnostics, chemical processing, and biomaterials, where they may incorporate features such as molecular recognition and enzymatic activity. Understanding the forces that direct self-assembly of biologically important molecules in predictable arrangements will aid the development of such applications. The focus of this study is a mimic of biological membranes formed by the interaction between two self-assembled systems: phospholipid amphiphiles that associate into bilayer vesicles in water, and monolayers of alkanethiols on metal surfaces. We have studied the interaction of small (60nm) POPC vesicles with binary thiol monolayers of known surface free energy. The surfaces were prepared on gold by self-assembly from binary solutions of the thiols CH3-(CH2)10-X (X = CH3; OH) in THF. The surface plasmon resonance (SPR) technique was utilized to follow the vesicle fusion kinetics and to characterize the resulting assemblies. A dramatic influence of the surface layer composition on the formation of POPC films was observed. The formation of an additonal POPC monolayer was detected only on the completely hydrophobic (100% CH3) surface. The largest thickness of POPC layer was detected at a CH3/ OH ratio of 50% (in the assembly solution). For the completely hydrophilic surface (100 % OH) the POPC layer thickness was found to be close to the thickness of a phospholipid bilayer. Thus, the increase of hydrophilic component on the surface leads to the formation of an unordered POPC film that seems to contain a mix of fused and unfused vesicles. Most likely the formation of an ordered bilayer of POPC molecules has been observed for the completely hydrophilic surface. The SPR data were supported by AFM, capacitance and contact angle measurements.

10:20am **BI+SS-MoM7 Formation of 2D Crystals of Proteins on Solid Supports, and Their Application for Immobilizing Molecules or Particles**, ***A.D.R. Brisson***, University of Groningen, The Netherlands **INVITED**

The immobilization of molecules or particles on solid supports constitutes a central issue in various fields, eg. the immobilization of enzymes in the biosensor area, or the immobilization of DNA molecules on microarrays in genomics. Existing technologies rely mainly on the chemical modification of solid surfaces and the subsequent immobilization of the molecules of interest via non-specific interactions. The strategy we have selected is based on the use of functionalized 2D crystals of proteins formed on solid-supported lipid bilayers (SPBs) as a matrix for anchoring proteins/particles in a specific manner. Its main potential advantages are the wide panoply of functional groups that could be introduced in proteins, the well-known chemistry of the coupling reactions involved, the well-defined density of anchoring groups, and the specificity of the coupling reactions ensuring an oriented binding of bound molecules. In addition, protein 2D crystals could serve as templates for creating ordered arrays of immobilized particles, at the nanometer scale. The formation of SPBs by fusion of lipid vesicles on mica,@footnote 1@ and the growth of protein 2D crystals on SPBs were extensively studied by AFM and Electron Microscopy (EM) in the case of two protein systems, annexin V@footnote 2@ and streptavidin. Pre-formed 2D arrays of modified annexin molecules were used for immobilizing proteins, liposomes, and membrane fragments containing membrane proteins. An unexpected result was the induced ordering of membrane proteins resulting from their specific binding to an ordered protein matrix. On the other hand, while close-packed assemblies of liposomes could be bound to protein 2D arrays, attempts to fuse them into suspended lipid bilayers have yet been unsuccessful. The immobilization of

inorganic particles is under investigation. Results of these studies will be presented. @FootnoteText@@footnote 1@Reviakine et al. Langmuir 16, 2000, 1806. @footnote 2@Reviakine et al. J. Struct. Biol. 121, 1998, 356.

Biomaterial Interfaces
Room 202 - Session BI+MC-MoA

Characterization of Biomaterial Interfaces

Moderator: L. Hanley, University of Illinois at Chicago

2:00pm **BI+MC-MoA1 Surface Tools for the Characterisation of Biomaterials**, ***M.C. Davies***, *S.J.B. Tendler, C.J. Roberts, P.M. Williams, S. Allen,* University of Nottingham, UK **INVITED**

Understanding the interfacial chemistry of biomaterials has long been a goal in the search for optimum biocompatibility. The interfacial environment has a major control on materials properties and the exploitation of nanosurface engineering, to tailor the optimum surface behaviour and function has made a significant impact in the biotechnological and biomedical sciences over the last decade. This talk will explore the role of advanced surface tools for the characterisation of modern biomaterial materials and review the limitations and advantages of different approaches, highlighting fruitful areas for future activity.

2:40pm **BI+MC-MoA3 Electrochemical SPR for Biomaterial Applications**, ***R.M. Georgiadis***, Boston University, usa; *R.J. Heaton,* Boston University

Electrochemical SPR is the combination two powerful surface specific techniques which together provide the basis for many surface modification and detection schemes with new applications to biomaterial interfaces. Although the general effect of an applied electrochemical potential on the surface plasmon resonance response from a simple metal surface is well known, the response from more complex interfaces has not been studied in detail. Yet, such studies are crucial for many applications such as quantifying binding at interfaces in the presence of an applied electrochemical field. We show that very useful information can be obtained directly from the potential dependent SPR optical data: the potential of zero charge can be determined in the presence or absence of immobilized organic or biomaterial films and the specific adsorption of anions can also be distinguished. We report results for a series of modified interfaces including self assembled monolayer films and for thiol bound DNA oligomer films in various electrolytes.

3:00pm **BI+MC-MoA4 Characterization of Surface Modified Microporous PTFE Biomembranes using Surface Charge, Topography and Chemistry Studies**, ***I.D. Baikie***, *B. Lägel,* Robert Gordon University, UK

Functionalised microporous PTFE membranes have many applications involving cell growth and adhesion such as skin grafting and cell scaffolds. Key factors in promoting cell growth are the chemistry and topography of the surface, however a much overlooked surface parameter is that of surface charge. Using a multitip scanning kelvin probe (SKP)@footnote 1@ we have performed surface potential/charge topographies of bare and surface modified bio-membranes prior to Human Skin Fibroblast growth. Additionally surface characterization with SEM and XPS provided topography and chemistry information on the top-most layers. Subsequent video-microscopy growth data indicates an extraordinary correlation between a regime of homogeneous negative surface charge profiles and confluent HSF films. Indeed the optimum growth surface displays two dimensional charge transport characteristics. Up to now little work has been performed on the electrical properties of modified polymers due to the difficulties in obtaining accurate surface potential data. The SKP features a truly noninvasive charge imaging measurement mode and we anticipate many future applications both in monitoring biomaterials and biological interfaces. @FootnoteText@ @footnote 1@I. Baikie, P.J.S. Smith, D.M. Porterfield and P.J. Estrup, Rev. Sci. Instrum. 70, 1842 (1999).

3:20pm **BI+MC-MoA5 Enhanced TOF-SIMS Imaging of a Micropatterned Protein by Stable Isotope Protein Labeling**, ***A. Chilkoti***, Duke University; *A. Belu,* Physical Electronics; *Z.P. Yang,* Imation Inc.; *R. Aslami,* Duke University

Patterning of biomolecules on surfaces is an increasingly important technological goal. Because the fabrication of biomolecule arrays often involves step-wise, spatially resolved derivatization of surfaces, spectroscopic imaging of these arrays is important in their fabrication and optimization. Although imaging time-of-flight secondary ion mass spectrometry (TOF-SIMS) is a powerful method for spatially resolved surface analysis of organic molecules on surfaces, TOF-SIMS images of micropatterned proteins on organic substrates can be difficult to acquire because of the lack of high intensity, protein specific molecular ions that are essential for imaging under static conditions. In contrast, low mass ions are of suitable intensity for imaging, but can originate from different chemical species on the surface. A potential solution to this problem is utilize stable-isotope labeled proteins, an approach that has heretofore not been explored in TOF-SIMS imaging of micropatterned proteins and peptides. In order to investigate the feasibility of stable isotope enhanced TOF-SIMS imaging of proteins, we synthesized @super 15@N-labeled streptavidin by metabolic labeling of the protein during expression from a recombinant gene. The spatial distribution of streptavidin bound to biotin micropatterns, fabricated on a polymer and on a self-assembled monolayer on gold, was imaged by TOF-SIMS. Imaging of high intensity, low m/z secondary ions (e.g., C@super 15@N@super -@ and C@super 15@NO@super -@) unique to streptavidin, enabled unambiguous spatial mapping of the micropatterned protein with a lateral resolution of a few microns. TOF-SIMS imaging of micropatterned @super 15@N-labeled streptavidin also illustrated the exquisite sensitivity of TOF-SIMS to low fractional coverage of protein (0.5 nm effective thickness) in the background regions of the protein micropattern.

3:40pm **BI+MC-MoA6 Quantitative Chemical Mapping of Lipid-protein Langmuir-Blodgett Layers by Laser-SNMS**, ***N. Bourdos***, *F. Kollmer, R. Kamischke, H.-J. Galla, A. Benninghoven,* Westfälische Wilhelms-Universität, Germany

Quantitative molecular mapping of chemically modified or functionalized surfaces is still an important challenge in surface analysis. We demonstrate that analyzing sputtered neutrals may be a big step forward in the quantitative mapping of laterally structured overlayers of organic molecules or biomolecules, respectively. We studied samples consisting of phospholipids and a small 34-residue peptide, the surfactant protein C (SP-C). These overlayers are phase-separated into a fluid and condensed phase. They were prepared on Au substrates with the Langmuir-Blodgett (LB) technique and investigated using a combined SIMS/SNMS instrument equipped with a reflectron-type TOF analyzer, a 30 keV Ga+ primary ion source, and an excimer laser for resonantly enhanced multiphoton post-ionization of neutrals. Laser-SNMS was applied for the first time to study LB layers. The SP-C clearly engenders typical amino acid-specific neutral fragments, by which it can be identified and localized on a surface. Most of them result from the cleavage of the COOH group, e. g., CH@sub4@N, C@sub 4@H@sub 8@N or C@sub 5@H@sub 12@N. The small CN is not typical of a certain amino acid but the entire molecule. It is the most intense peptide-based secondary particle and therefore gives excellent maps of SP-C-rich domains formed in the overlayer. It is possible to calculate the protein content in a lipid layer by histogram evaluation. The yields and damage cross-sections calculated from TOF-SIMS measurents indicate that the lateral resolution may be far below instrument limitations (beam focus). A quantitative comparison of the secondary particle emission from SP-C-rich and SP-C-free domains (on the same substrate) allowed some insight into the process of secondary ion and neutral generation from the molecular overlayer as well as from the substrate.

4:00pm **BI+MC-MoA7 Probing the Spatial Organization of Mixed Lipopeptide/Phospholipid Monolayers : Complementarity of AFM and XPS**, ***Y.F. Dufrene***, Universite Catholique de Louvain, Belgium; *M. Deleu, P. Jacques,* Faculte Universitaire des Sciences Agronomiques de Gembloux, Belgium; *P. Thonart,* Centre Wallon de Biologie Industrielle, Belgium; *M. Paquot,* Faculte Universitaire des Sciences Agronomiques de Gembloux, Belgium

Surfactin is a surface-active bacterial lipopeptide, with important biological properties, which is known to interact with lipid membranes. To gain insight into the spatial organization (miscibility, molecular orientation) of mixed surfactin/dipalmitoyl phosphatidylcholine (DPPC) monolayers, the morphology and chemical composition of mixed monolayers transferred on mica were determined by atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS), respectively. AFM topographic and friction images revealed phase-separation for mixed monolayers prepared at 0.1, 0.25 and 0.5 surfactin molar ratios. The step height measured between the surfactin and the DPPC domains was about 1.2 nm, pointing to a difference in molecular orientation: while DPPC had a vertical orientation, the large peptide ring of surfactin was lying on the mica surface. These data were in excellent agreement with the monolayer properties at the air-water interface and with computer simulation data. The N/C atom concentration ratios obtained by XPS for pure monolayers were consistent with two distinct geometric models: a random layer for surfactin and for DPPC, a layer of vertically-oriented molecules in which the polar headgroups are in contact with mica. XPS data for mixed systems were accounted for by a combination of the two pure monolayers, considering respective surface coverages that were in excellent agreement with those measured by AFM. Finally, exciting new possibilities offered by dynamic AFM imaging modes (force modulation, phase imaging) to

investigate the film nanomechanical properties will be presented. This work demonstrates the complementarity of AFM imaging and XPS analysis to directly probe the molecular organization of multicomponent monolayers.

4:20pm **BI+MC-MoA8 Detection of Intact Biomolecules with Matrix-Enhanced ToF-SIMS**, ***D.G. Castner***, *P. Kingshott, J. Nesiba, S.L. Golledge, B.D. Ratner,* University of Washington

An improved understanding of the interfacial interactions between biomolecules and surfaces is important for the successful design of the next generation of biomaterials. This study combines the high surface sensitivity and chemical specificity of ToF-SIMS with the 'soft' ionization capabilities of MALDI. Model peptides with beta-sheet and alpha-helix structures were used in conjunction with MALDI matrix molecules [2,5-dihydroxybenzoic acid (DHB) and sinapinic acid (SA)] to facilitate generation of molecular ions with the SIMS Cs+ ion source. The positive ToF-SIMS spectra from the beta-sheet peptide incorporated into crystals of DHB show peaks representative of sodium-adduct ions of the peptide (M-H+Na+) (m/z 1096.7). The spectrum from the bulk beta-sheet contains only fragment ions and no molecular ions, suggesting that there is a synergistic effect in producing sodium adduct molecular ions when both Na and matrix molecules are present. When sodium is eliminated from the system, peaks that can be assigned to the M+ ion (m/z 1074.7) can still be detected. Molecular ions from the alpha-helix peptide were also detected when DHB was present. The use of SA as matrix failed to generate peptide molecular ions, suggesting the matrix-specific nature of this MALDI-SIMS technique. Imaging-SIMS indicated that the peptides are incorporated within the DHB matrix crystals, but are not fully incorporated into the SA crystals. This shows the importance of good mixing between the peptides and matrix molecules for detection of intact molecular ions.

4:40pm **BI+MC-MoA9 Molecular Orientation of Annealed Artificial Joint Polymers: Characterization by Soft X-ray Absorption**, *S. Sambasivan,* SUNY Stony Brook; ***D.A. Fischer***, National Institute of Standards and Technology; *M. Shen,* University of Maryland; *J.A. Tesk, S. Hsu,* National Institute of Standards and Technology

For the past 30 years ultra-high molecular weight polyethylene (UHMWPE) has remained the dominant polymer in artificial joints due to its outstanding wear resistance properties. It has been recognized that wear of UHMWPE contributes to the loosening of the implants and is the main cause for the failure of long-term implants. Hence, there is an urgent need to understand the mechanism and the surface morphology leading to wear and failure of the artificial joint. Hubbard et al.@footnote 1@ and Hastings et al.@footnote 2@ have demonstrated that the molding and annealing the UHMWPE at safe elevated temperatures resulted in increased mechanical strength. Also, cross-linking of UHMWPE has been demonstrated to reduce wear significantly. We have previously measured molecular orientation as a function of wear motion.@footnote 3@ Molecular orientation in biomaterials is thought to be critical in characterizing the precursors of wear and the production of debris during the wear process. While the link between molecular orientation and wear has not been clearly established, molecular orientation has been recognized as an important parameter in wear resistance. This study examines the change of molecular orientation caused by annealing UHMWPE. Our technique utilizes soft x-ray absorption spectroscopy at a synchrotron beamline to non-destructively characterize the molecular orientation of the UHMWPE surface layer. Current methods of inferring or deducing orientation are not accurate and often rely on staining and cutting specimens.@FootnoteText@ @footnote 1@Hubbard et al., Trans. 25th Soc. For Biomaterials, 325(1999). @footnote 2@Hastings et al., Trans. 25th Soc. For Biomaterials, 328(1999). @footnote 3@Fischer et al., Trans. 25th Soc. For Biomaterials, 351(1999).

5:00pm **BI+MC-MoA10 Titanium-Alginic Acid Chemistry of Adhesion Using X-ray Photoelectron Spectroscopy**, ***R.A. Brizzolara***, David Taylor Research Center, NSWC

The interfacial chemistry between alginic acid and a titanium surface has been examined using x-ray photoelectron spectroscopy (XPS). This study is motivated by the effort to mitigate effects of seawater biofouling on heat transfer surfaces via materials or surface modification strategies. Alginic acid is a predominant adhesive in bacterial biofilms, and titanium is a common material in naval ship cooling and piping systems. XPS has been used to quantify the alginic acid adsorbed to the titanium surface from aqueous solution. The experiments were performed at various solution pH's to examine the effect on alginic acid adsorption of changing the charge state of the ionizable groups on the alginic acid and of the titanium surface. The effects of ions in the solution were investigated by performing the alginic acid adsorption in the presence of calcium chloride. To separate the effects of the carboxyl and hydroxyl moieties present in alginic acid, XPS has also been used to measure caproic acid (carboxyl) and glucose (hydroxyl) adsorption as a function of solution pH. High-resolution XPS spectra have been utilized to separate the various carbon and oxygen chemistries present, and angle-resolved XPS spectra and advancing contact angle measurements were used to elucidate molecular orientation effects. Atomic force microscope (AFM) images were obtained to determine adsorbate morphology and surface coverage. These data will be interpreted in light of potential alginic acid - titanium adsorption mechanisms such as hydrogen bonding and anion exchange. This information regarding the biofilm-surface chemical interaction will be useful in developing fouling resistant surfaces. The NSWC Carderock Division In-House Laboratory Independent Research Program supported this work.

Biomaterial Interfaces
Room 202 - Session BI-TuM

Protein-Surface Interactions

Moderator: B.D. Ratner, University of Washington

8:20am **BI-TuM1 Quantitative Analysis of Protein Adsorption Kinetics*, V. Hlady,*** University of Utah **INVITED**

Protein adsorption from aqueous solution is determined by a "match" between two interfaces, one between the protein and the aqueous solution and the other between the adsorbent surface and the solution. A subtle interplay between polar and non-polar interactions regulates protein stability and plays a decisive role in protein interactions with the adsorbent surface. Other factors include the adsorbent's surface energetics, charge, rugosity, and the structure of water at both interfaces, i.e. their respective hydrophilicity and interfacial hydration layers. In order to characterize and predict protein adsorption, one seeks information about adsorption isotherms and kinetics, conformation of adsorbed proteins, number and character of surface-bound pr otein segments, and the physical parameters describing the adsorbed protein layer. The most powerful techniques for protein adsorption studies include optical and spectroscopic methods. These methods can provide insight into protein concentration, confor m ation and dynamics at interfaces. We have designed a spatially-resolved total internal reflection fluorescence spectroscopy method (1-D TIRF) to measure competitive adsorption kinetics of human plasma proteins. When combined with autoradiography and sur fa ce hydrophobicity gradients, 1-D TIRF experiments provide a quantitative description of protein adsorption and desorption kinetics as a function of surface hydrophobicity. As an example we will show the analysis of the adsorption kinetics from a binary so lution mixture of human serum albumin (HSA) and human low density lipoproteins (LDL) onto the model surface with a density gradient of octadecyldimethylsilyl chains on fused silica (C18-silica gradient). The adsorption and desorption rate constants are obtained by fitting the experimental results to an adsorption model that accounts for the mass transport effects and the surface density of the C18 groups.

9:00am **BI-TuM3 Investigation of the Structure and Dynamic of Proteins on Surfaces by EPR Spectroscopy: Annexin XII as an Exploratory Example*, T. Risse,*** *W.L. Hubbell,* University of California, Los Angeles*; M. Isas, H. Haigler,* University of California, Irvine

Site-directed spin labeling (SDSL) has become an important tool for the investigation of structure and dynamics in proteins. The SDSL strategy involves introduction of one or two nitroxide side chains (R1) at selected positions in the protein sequence, followed by analysis of the electron paramagnetic resonance (EPR) spectrum in terms of secondary and tertiary structure. To increase the information content of the SDSL experiment, and to examine protein structure and dynamics at interfaces, oriented arrays of spin-labeled proteins on surfaces are now under investigation. Initial experiments have employed the helical protein Annexin XII adsorbed to single lipid bilayers supported on glass or mica surfaces. For molecules oriented in 2-dimensions, the tensorial nature of the Hamilton operator gives rise to angular dependent EPR spectra which can be used to extract the orientation of the nitroxide relative to the surface. Such information is of paramount importance for determining the topography of proteins bound to surfaces. In addition, direct information on the structure and interactions of the protein at the surface is obtained from the dynamics of the side chains inferred from the spectral lineshape. Results for R1 residues at sites 213, 147, 148 and 154, 156 in oriented monolayers of Annexin XII will be discussed relative to these points.

9:20am **BI-TuM4 A Surface Plasmon Resonance Biosensor Study of Protein Interactions with Thin Apatite Films*, H.B. Lu,*** University of Washington*; B.J. Tarasevich,* Battelle Pacific Northwest National Laboratory*; C.T. Campbell, C. Giachelli, B.D. Ratner,* University of Washington

The primary objective of this research is to study protein interaction with a well-characterized apatite film using a surface plasmon resonance (SPR) sensor. Due to the remarkable osteointegration properties of apatite as an implant material, protein adsorption onto this type of material has been intensively studied. It is believed that knowledge on protein adsorption to such apatite surface will help us to understand the body's response to foreign materials and improve implant performances. However, due to the limitations of analytical tools for either material characterization or protein adsorption, few useful conclusions have been reached. In this study, a gold SPR sensor has been functionalized with a carboxylic acid-terminated self-assembled monolayer and then coated with an apatite thin film grown with the surface-induced mineralization technique. The apatite mineral films have been well characterized using surface analytical tools including TOF-SIMS, XPS, FTIR, and AFM, as well as bulk analytical technique XRD. SPR is an optical phenomenon that is very sensitive to refractive index changes perturbing the evanescent wave at metal-liquid interfaces. By coating the SPR sensor with a thin apatite film, we took advantages of continuous and label-free monitoring, and thus studied protein interactions with apatite in real time. The proteins studied include phosphorylated Rat His osteopontin (p-OPN), non-phosphorylated Rat His osteopontin (n-OPN), and bovine serum albumin (BSA). The p-OPN displayed very distinguishable adsorption/desorption behavior from that of n-OPN and BSA. The p-OPN has a higher affinity toward the apatite surface, compared to n-OPN and BSA. The p-OPN may have inhibited mineral growth upon adsorption, while BSA may have promoted slight mineral growth upon adsorption. On the other hand, these proteins behaved non-selectively when adsorbing onto control surfaces including bare gold, a COOH-terminated SAM and an NH2-terminated SAM.

9:40am **BI-TuM5 Protein Adsorption to Plasma Functionalized Surfaces Using Surface Plasmon Resonance Spectroscopy and Atomic Force Microscopy*, M.T. van Os,*** University of Twente*; A.T.A. Jenkins,* Max Planck Inst. for Polymer Res.*; M. Péter,* University of Twente*; R. Förch,* Max Planck Inst. for Polymer Res.*; R.B. Timmons,* The Univ. of Texas at Arlington*; W. Knoll,* Max Planck Inst. for Polymer Res., Germany*; G.J. Vancso,* University of Twente, The Netherlands

Plasma modification provides a powerful tool to tailor the surface properties of materials. Surface characteristics such as wettability, chemistry and morphology are known to influence protein adsorption, and the subsequent attachment and spreading of cells on biomaterials. To improve the understanding of protein-surface interactions we functionalized gold and silicon surfaces with amino or ether groups, using radio frequency plasma polymerization of ethylenediamine, allylamine, cycloheptylamine and di(ethyleneglycol)vinylether (EO2V). The functional group density at the surface was controlled by using different monomers or by variation of the input power during the plasma deposition. The adsorption of the proteins fibrinogen, bovine serum albumin and immunoglobulin G to these surfaces was measured in situ with surface plasmon resonace spectroscopy. The tenacity of the protein adsorption on the different substrates was also measured, after removing elutable protein with 1% sodium dodecyl sulfate (SDS) solution. After drying, the protein layers were studied by tapping mode atomic force microscopy (TM-AFM). The results obtained show that both the protein adsorption to and the retention on the surfaces are affected greatly by the surface functionalities. All the amine functionalized surfaces showed a high affinity toward the proteins, and thin dense layers of adsorbed protein remained on these surfaces, even after rinsing with SDS solution. A large contrast in protein affinity was observed between the EO2V films polymerized at different power input conditions. A dramatic reduction in both initial adsorption and retention of all proteins was observed on these films with decreasing power. The low degree of cross-linking, as well as the high retention of ether content during the polymerization of EO2V under low power input conditions is thought to result in the production of biologically non-fouling surfaces.

10:00am **BI-TuM6 How to Make and Analyze Cross-linked Monolayers of Mytilus Edulis Foot Proteins (Mefp)*, H. Elwing,*** *K. Mjorn,* Lab of Interface Biophysics, Sweden*; K. Uvdal, M. Fahlman,* Linkoping University, Sweden*; J Lausmaa,* National Testing and Res. Institute, Sweden*; F. Hook,* Lab of Interface Biophysics, Sweden

The Mefp proteins are potential candidates as "tissue glues" in biomaterial applications. Several of the Mepf proteins contain high amounts of DOPA (dihydroxyphenylalanin). On oxidation to o-quinone the DOPA molecules become highly reactive and forms a base for cross-linking of the proteins in the byssus threads as well as binding to solid surfaces. We have developed an experimental model consisting of polar siliconoxide surfaces and apolar alkanethiol surfaces. On this surfaces we follow adsorption of purified Mefp-1 with the use of optical methods such as surface plasmon resonance (SPR) and high precision ellipsometry. Periodate induced crosslinking of the molecular layers is then followed by Quarts crystal microbalance (QCM-D) and ellipsometry. At apolar surfaces we found a reduction of layer thickness from about 20 nm to about 4 nm as well as a significant reduction of the viscoelastic properties of the protein layers as measured by QCM-D. On the other hand, adsorption of Mefp-1 on polar surfaces res ulted in a protein layer that was thin and dense from the beginning and cross-linking resulted only in significant small change of layer thickness and

viscoelasticity of the mefp-1 layer. It was obvious that adsorption of Mefp-1 to polar surfaces significantly reduced the possibility of cross-linking most probably due to binding engagement of the DOPA side chains to the silicon oxide surface. We also made an analysis with photoelectron spectroscopy (XPS). High-resolution spectra at normal and glancing take off angles were obtained with a particular emphasis placed on the C 1s core level. Four distinct peaks were visible in the non-deconvoluted spectrum. Carbon-nitrogen and carbon-oxygen bonds were studied as a function of depth, crosslinking and the polarity of the substrate. Significant differences were found and is now subjected to a more detailed analysis together with data from time of flight secondary ion spectroscopy (TOF-SIMS).

10:20am **BI-TuM7 Assessment of Fibronectin Conformation Adsorbed to Polytetrafluoroethylene Surfaces from Serum Protein Mixtures and Correlation to Support of Cell Attachment in Culture**, ***D.W. Grainger,*** Colorado State University; *G. Pavon-Djavid, V. Migonney, M. Josefowicz,* Universite Paris, France

Fluoropolymer surfaces in biotechnology applications are notorious for tightly adsorbing proteins that do not support cell attachment. Reasons for this remain confusing but surround both the population and conformation of proteins adsorbed competitively from physiological milieu that do not interact with cell adhesion receptors. In this study, polytetrafluoroethylene (PTFE) surfaces were exposed to buffered aqueous solutions containing radio labeled (@super 125@I) human fibronectin (Fn), fibronectin:serum albumin (BSA) binary mixtures of various ratios, or whole human plasma dilutions (one hour). Total adsorbed fibronectin and albumin following rinsing were quantified on PTFE. @super 125@I-labeled monoclonal antibodies against either the fibronectin cell adhesion (containing the RGDS integrin recognition motif) or the fibronectin amino terminal domains were used to probe accessibility of each of these fibronectin regions post-adsorption. Human umbilical vein endothelial cells (HUVECs) were then cultured on PTFE surfaces pre-exposed to each of these protein adsorption conditions and compared to identical conditions on tissue culture polystyrene (TCPS) controls. Fibronectin adsorption to PTFE is dependent upon the concentration of albumin co-adsorbing from solution: albumin out-competes fibronectin for PTFE surface sites even at elevated nonphysiological Fn:HSA ratios. Antibodies against Fn do not readily recognize Fn adsorbed on PTFE as the HSA co-adsorption concentration in either binary mixtures or in plasma increases, indicating albumin masking of adsorbed Fn. At higher Fn:HSA ratios, albumin co-adsorption actually improves anti-Fn antibody recognition of adsorbed Fn. HUVEC attachment efficiency to PTFE after protein adsorption correlates with amounts of Fn adsorbed and levels of anti-Fn antibody recognition of Fn on PTFE, linking cell attachment to integrin recognition of adsorbed Fn density and Fn adsorbed conformation on PTFE surfaces.

10:40am **BI-TuM8 Protein Adsorption on Self-assembled Polyelectrolyte Multilayer Films**, *G.D. Ladam,* Institut Charles Sadron, France; *F.J.G. Cuisinier,* Federation de Recherche "Odontologie", France; *G. Decher,* Institut Charles Sadron, France; *J. Voegel,* Federation de Recherche "Odontologie", France; ***P. Schaaf,*** Institut Charles Sadron, France

Alternating polyelectrolyte films were constructed by the sequential adsorption of poly(allylamine hydrochloride) (PAH) and polystyrenesulfonate (PSS) onto a silica surface. The film build-up and the further adsorption of proteins (human serum albumin (HSA), ribonuclease A, lysozyme, alpha-lactalbumine, myoglobine) were followed in situ versus time by means of scanning angle reflectometry. We investigated first the influence of the isoelectric point of the proteins on their adsorption onto positive (PAH ending) and negative (PSS ending) multilayers. At a protein concentration of 0.25mg/ml at pH 7.4 and in the presence of Tris HCl 10-2M, 0.15M NaCl buffer, all proteins adsorbed on both positive and negative polyelectrolyte films with thicknesses varying from the monolayer or less, up to thicknesses equivalent to at least 4 protein layers. Thick protein layers were observed when proteins and films were oppositely charged. The adsorption of HAS onto both films was investigated as a function of the protein concentration and the NaCl concentration of the adsorbing solution. It was found that on PSS ending multilayers exhibiting a similar charge as albumin, the proteins still adsorb but only a monolayer can be reached. On the other hand, on PAH ending multilayers thick protein films are observed. The adsorbed amount depends also critically on the NaCl concentration of the adsorbing solution. Desorption experiments were also performed and depending on the salt concentration of the rinsing solution one can observe no desorption or partial desorption (up to 50% of the adsorbed amount). A microsocopic model will be discussed trying to explain these experimental findings.

11:00am **BI-TuM9 Desorption/Ionization Mass Spectrometry on Porous Silicon Surfaces**, ***Z. Shen,*** *J.E. Crowell,* University of California, San Diego; *G. Siuzdak,* The Scripps Research Institute

A new desorption/ionization strategy for biomolecular mass spectrometry has been developed based on pulsed laser desorption/ionization from a porous silicon surface. Desorption/ionization on silicon (DIOS) uses porous silicon to trap analytes deposited on the surface and laser radiation to vaporize and ionize these molecules. DIOS is demonstrated for a wide range of small molecules as well as biomolecules at the femtomole and attomole level with little or no fragmentation, in contrast to what is typically observed with other direct desorption/ionization approaches. Porous silicon surfaces were prepared using electrochemical etching. While DIOS has been universally applicable for a range of mass analyses, its success is highly dependent upon the preparation of the sample and the nature of the porous silicon surface. Different etching parameters, including silicon wafer crystal orientation, dopant type, dopant level, light intensity, current density, etching solution, and etching time were studied to optimize DIOS-MS performance. Scanning Electron Microscopy (SEM) was used to examine the pore structure and correlate it with DIOS-MS performance. We will also demonstrate the application of DIOS-MS to small molecule analysis and quantitation, protein identification, on-chip reaction monitoring, on-chip separation and post-source decay structure analysis. DIOS offers many unique advantages including good sensitivity, low background ion interference, and high salt tolerance. Desorption/ionization on porous silicon (DIOS) permits analysis of a wide range of molecules with very good sensitivity and a demonstrated potential for automation, as well as compatibility with microfluidics and microchip technology on silicon.

11:20am **BI-TuM10 Determination of Surface-Protein Equilibrium Binding Constants by MALDI Mass Spectrometry**, ***G.R. Kinsel,*** *J. Zhang, R.B. Timmons, H. Qiu,* University of Texas at Arlington

We have recently demonstrated that matrix assisted laser desorption / ionization (MALDI) mass spectrometry offers a new approach for the characterization of surface-protein interactions. Our work demonstrates that strongly surface-retained proteins are poorly incorporated into the MALDI matrix crystals, leading to inefficient ionization of these species. In effect, the surface-deposited protein MALDI ion signal approaches zero as the quantity of deposited protein approaches the quantity strongly retained by the surface. Furthermore, as expected, the protein MALDI ion signals exhibit Langmuir type behavior as the surface concentration of the protein is reduced, i.e. the protein MALDI ion signal versus surface concentration response becomes asymptotic at low protein surface concentrations. Analysis of the protein MALDI ion signal versus surface concentration data allows the equilibrium surface-protein binding constant to be established. In our current work this approach to the determination of surface-protein binding constants has been applied to a number of smaller peptides and proteins deposited on a variety of polymeric biomaterials. Expected trends are observed, particularly with regard to the influence of electrostatic interactions between acidic or basic surfaces and basic or acidic proteins in solution. In addition, we have used the MALDI approach to examine the elutability of surface-bound proteins as a function of solvent choice. Our studies indicate that protein solubilization as a function of solvent choice is strongly influenced by the chemistry of the surface-protein interaction.

11:40am **BI-TuM11 The Molecular Orientation Distribution of an Electrochemically Active Protein Monolayer Adsorbed to Indium-Tin Oxide**, *S. Saavedra,* ***R.T. Robertson,*** *S.B. Mendes, N.R. Armstrong,* University of Arizona

The relationship between molecular orientation and heterogeneous electron transfer behavior in immobilized films of redox-active proteins is being investigated using absorbance and fluorescence techniques that combine the information content of spectroele ctrochemistry with the sensitivity of the single-mode, planar waveguide geometry. Spectroelectrochemistry of surface confined, redox-active films can be performed with a pathlength enhancement of approximately 4,000 relative to a transmission geometry. T he use of this approach to determine the tilt angle distribution of the porphyrin molecular planes in a submonolayer of electrochemically active cytochrome c adsorbed to an indium-tin oxide electrode will be described. Developing a better understanding of the relationship between protein film structure and redox activity may aid efforts to rationally design protein-based molecular devices in which control of vectorial electron transfer is a prerequisite for efficient operation.

Surface Science
Room 209 - Session SS2+NS+BI+EL-TuM
Self-Assembled Monolayers

Moderator: D.H. Fairbrother, Johns Hopkins University

8:20am **SS2+NS+BI+EL-TuM1 Creating Highly Selective Organic Surfaces using Self-assembly: A New Family of Organothiols**, ***R. Arnold,*** Ruhr-Universität Bochum, Germany*; A. Terfort,* Universität Hamburg, Germany*; C. Wöll,* Ruhr-Universität Bochum, Germany

The creation of organic surfaces with specific properties via the adsorption of correspondingly functionalized organothiols has recently attracted considerable interest, e.g. in the context of bio-sensors and biomimetics. In case of alkanethiols some functional groups, however, interact so strongly with each other that the ordering within the SAMs is affected, e.g. in the case of -COOH functional groups.@footnote 1@ The situation can be improved by using more rigid backbones, e.g. oligophenyl units.@footnote 2@ With regard to biochemical applications in many cases the distance between adjacent organothiol units (4.97 Å) is too small to immobilize larger molecules, e.g. small proteins. In the past these problems could be overcome in some cases by diluting the functionalized organothiol in a shorter, nonfunctionalized thiol. Here, we present a different approach where a more bulky thiol is used, which increases the nearest neighbor distance. We will present the results of a study using several homologues of triptycenethiols. SAMs formed from these compounds were characterized by using XPS, IRRAS, NEXAFS, LEED and TDS. The results reveal the formation of well ordered monolayers, which are anchored to the gold surface in a more distant lateral structure than alkane- or p-terphenylthiols. @FootnoteText@ @footnote 1@ Dannenberger, O.; Weiss, K.; Himmel, H.-J.; Jäger, B.; Buck, M.; Wöll, C. Thin Solid Films 1997, 307, 9885-9893 @footnote 2@ Himmel, H.-J.; Terfort, A.; Wöll, C. J. Am. Chem. Soc. 1998, 120, 12069-12074.

8:40am **SS2+NS+BI+EL-TuM2 Characterization of the Alkanthiol/Metal Interface by High Resolution Core Level Spectroscopy**, ***K. Heister,*** *H. Rong, M. Buck,* University Heidelberg, Germany*; L. Johansson,* University Karlstad, Sweden*; M. Zharnikov, M. Grunze,* University Heidelberg, Germany

During the last decade X-ray Photoelectron Spectroscopy with a laboratory X-ray source became a conventional technique to characterize thiol derived SAMs. However, due to the mostly poor energy resolution, a strong attenuation of the photoelectron signal, and a low photoionization cross-section of the relevant core levels at high photon energies a precise binding energy analysis of an important building block of a SAM, the SAM/metal interface was hardly possible, even though high resolution photoelectron spectroscopy could give important information about the chemical state of the atoms in this region. Taking advantage of the high performance and tunebility of the third generation synchrotron sources we have firstly applied the synchrotron-based High Resolution Core Level Spectroscopy to study the SAM/metal interface. The variable photon energy of the synchrotron light and a high energy resolution of the spectrometer (0.1-0.3 eV) enabled us to resolve the bulk and surface components of the substrate emission peak (Au 4f / Ag 3d) and monitor the evolution of these components upon the alkanethiol and biphenylthiol adsorption. Simultaneously, the interaction of the thiol-derived molecules with the substrate was followed by monitoring the S2p doublet attributed to the sulfur head group of these molecules. Only one sulfur species was found in the densely packed SAMs, which implies an equivalent bonding geometry for all adsorbed molecules. In SAMs comprising of specially designed, mixed aliphatic-aromatic molecules a periodical, 'odd-even' shift of the S2p binding energy with the varying length of the aliphatic part was observed. This shift can be attributed to the distortion of the substrate-S bonding angle resulting from the unfavorable package conditions occurring at definite lengths of the aliphatic part. This work has been supported by the German Bundesministerium für Bildung, Wissenschaft und Technologie through grant No. 05 SL8VHA 2 and by DAAD.

9:00am **SS2+NS+BI+EL-TuM3 The Influence of the Endgroup and the Chain Length on the Growth of CH@sub 3@- and CF@sub 3@-terminated Alkanetiols on Au(111)**, ***J. Pflaum,*** Princeton University*; G. Bracco,* University of Genova, Italy*; G. Scoles,* Princeton University*; R. Lee,* University of Houston*; A. Kahn,* Princeton University

The influence of the functional endgroup and the CH@sub 2@ chain length on the growth of alkanethiols on Au(111) was studied by scanning tunneling microscopy (STM) and x-ray surface diffraction in grazing-incidence geometry (GIXD). Thiols are model systems for self-assembled monolayers (SAMs) and exhibit a complex phase diagram as function of coverage. The structure and the electronic properties of the SAMs are determined by the sulfur headgroup, the CH@sub 2@ backbone and the functional endgroup. Leaving the sulfur headgroup unchanged, we studied how the film structure depends on the endgroup by comparing CH@sub 3@- and CF@sub 3@-terminated thiols. All films were prepared from solution on an atomically ordered Au(111) surface. The lateral order of the as-grown CH@sub 3@-terminated films corresponds to the c(4x2) phase, i.e. the highest density standing-up phase. From STM studies we conclude that the arrangement of CH@sub 3@ endgroups corresponds to a pinwheel-like structure rather than to a zig-zag-like structure. In contrast, CF@sub 3@(CH@sub 2@)@sub 9@SH showed no lateral ordering as seen by STM and GIXD. However, the difference between electron densities at the CF@sub 3@/vacuum and the SH/Au interfaces induces an oscillation of the GIXD reflectivity. Fitting the periodicity of the modulation using the Parratt formalism leads to an estimate of the film thickness and its roughnesses at both interfaces. In spite of the lack of lateral order the film appears to be made by standing-up molecules. Differences between the thickness measured by ellipsometry and x-rays will be discussed. We will also explore the lying-down phase of alkanethiols and fluorinated thiols as organic templates for organic heterostructures. Initial results on the growth of PTCDA on such templates will be presented. This work was supported by the MRSEC program of the National Science Foundation (DMR-9809483). J.P. thanks the Deutsche Forschungsgemeinschaft for support (Grant No. PF 385/1-1).

9:20am **SS2+NS+BI+EL-TuM4 Self-Assembled Monolayers of Terphenyl Derivatized Thiols; Adsorption, Insertion Process and Electrical Conduction**, ***T. Ishida,*** JRCAT-NAIR and PRESTO-JST, Japan*; W. Mizutani,* JRCAT-NAIR, Japan*; N. Choi,* JRCAT-ATP, Japan*; H. Tokumoto,* JRCAT-NAIR, Japan

The investigation of SAMs made from conjugated molecules is highly activated from a viewpoint of molecular electronics as well as stable SAM formation. In the present study, we have investigated an insertion process of conjugated molecules, terphenylthiol (TP0), terphenyl methanethiol (TP1), terphenyl propanethiol (TP3), into nonanethiol self-assembled monolayers (SAMs) on Au(111) by STM. STM observation revealed that the insertion process is dependent on the molecular length of conjugated molecules. At the initial stage of insertion, replaced area of TP1 is larger than those of TP0 and TP3. However, when the immersing time is more than 12h, the replaced area of TP3 is larger than those of TP0 and TP1. The insertion process is likely to be determined by the solubility of the conjugated molecules and thermal dynamics. The single molecular resistance were increased with the number of the methylene groups, and obtained about 20G-ohm (TP0), 40 G-ohm (TP1) and 66 G-ohm (TP3). The vertical conduction of the conjugated molecular domains of TP1 and TP3 depended on their lateral sizes, while strong dependence was not observed in the case of TP0, suggesting that methylene group is necessary between the sulfur and aromatic rings to increase the vertical conduction of molecular domain.

9:40am **SS2+NS+BI+EL-TuM5 Structure and Chemistry of Alkanethiol Self-Assembled Monolayers**, ***G.E. Poirier,*** *T.M. Herne, C.C. Miller, M.J. Tarlov,* National Institute of Standards and Technology **INVITED**

Derivatized alkanethiols form dense, physically blocking films on Au surfaces thereby providing an effective and parsimonious method to control the chemical, physical, and electron-transfer properties of electrode surfaces. To predict the function of these monolayers in device applications, scientist require an understanding the molecular-scale structure and chemistry. Our structure studies were conducted using gas-phase transport of decanethiol onto clean Au(111). Characterization was accomplished using ultrahigh vacuum scanning tunneling microscopy. At low surface coverage, decanethiol exists as a 2-dimensional gas. With increasing coverage the molecules sequentially condense into islands of three discrete commensurate crystalline lattices, each characterized by alignment of the molecular axes with the surface plane (striped phases). Above saturation coverage of the densest striped phase, the monolayer undergoes an edge-mediated melting transition forming a supercooled 2-dimensional liquid. Domains of the c(3x2*3) phase, characterized by alignment of the molecular axes close to the surface normal, nucleate and grow from this surface liquid. The reaction of these monolayers with ozone was characterized using scanning tunneling microscopy and x-ray photoelectron microscopy; our results show that exposure to ozone results in oxidation of the thiol terminus. The reaction initiates at the c(3x2*3) domain boundary network and propagates into the domains. Above a threshold surface oxygen content, the monolayer converts to a two-dimensional fluid that can subsequently recrystallize to a commensurate

monolayer of partially oxidized thiol. Further exposure to ozone results in conversion of the monolayer to a fluid phase and a 10% to 30% expansion of the Au lattice at the Au-thiol interface with concomitant formation of Au islands. Our results demonstrate that crystallographic defects in monolayer films can play an important role in their chemical reactions.

10:20am **SS2+NS+BI+EL-TuM7 Characterization of SAMs with Contrast Variation SPR Technique**, ***K. Tamada,*** NIMC and RIKEN Frontier Program, Japan; *H. Akiyama, J. Nagasawa,* NIMC, Japan

We report characteristics of azobenzene-containing self-assembled monolayers (SAMs) which is designed and synthesized for surface photo-isomerization reaction. The surface reaction was monitored by Surface Plasmon Resonance Spectroscopy (SPR), in which the contrast variation technique with various organic solvents was used to improve the accuracy for determination of the optical thickness change by surface reaction. The SAM formation was monitored by kinetic mode experiment with SPR in 0.1mM hexane solution, and after rinsing, refractivity change by UV-VIS photo irradiation was studied in various solvents. In this study, hexyl azobenzene thiol (12-(4-((hexylphenyl)azo)phenoxy)dodecane-1-thiol) SAM was used as an unreactive surface and unsymmetrical azobenzene-disulfides SAMs with the same azobenzene functions were used as reactive ones. Following the previous reports, monomeric dispersion of dye function with disordered chains seems to be necessary to realize highly reactive surface. For our unsymmetrical azobenzene-disulfides SAMs, the free volume for photo-isomerization reaction are guaranteed by 50% dilution of dye functions on surface at monomolecular level. As a result, it was confirmed that unsymmetrical azobenzene-disulfides SAMs were highly reactive, especially, in good solvents (alkanes: C5, C6, C7, C8) and the length of alkyl side chains was quite efficient for surface reactivity.

10:40am **SS2+NS+BI+EL-TuM8 Characterization of a Polymerized Self-Assembled Monolayer Using NEXAFS**, *A.L. Marsh, D.J. Burnett,* University of Michigan; *D.A. Fischer,* National Institute of Standards and Technology; *C.E. Evans,* ***J.L. Gland,*** University of Michigan

Near-edge X-ray Absorption Fine Structure, or NEXAFS, at the C-K-edge was used to characterize the orientation of the polymeric backbone in a self-assembled monolayer of 15,9-polydiacetylene. Monolayers were fabricated from the assembly of molecules of dinonacosa-10, 12-diyn-disulfide from a chloroform solution onto a 2000 Angstrom gold film on a mica substrate. Polymerization occurs across one of the C-C triple bonds in the chain, which results in a polymeric network located within the monolayer. Since resonance intensities in NEXAFS spectra are dependent on electric dipole selection rules, it is possible to determine the orientation of the polymeric backbone by comparing spectra at normal incidence (E vector parallel to the surface plane) with spectra at glancing incidence (E vector perpendicular to the surface plane). From the two spectra it was determined that the polymeric backbone is oriented parallel to the surface, while the alkyl chains are oriented perpendicular to the surface. Since NEXAFS probes unfilled molecular orbitals, it is capable of distinguishing between various bonds, i.e. a C-C double bond versus a C-C triple bond, making it possible to determine structural changes as a function of temperature. Upon increasing the temperature, the C-C double bond pi* resonance increases, while the C-C triple bond pi* resonance decreases. These changes would be consistent with a degradation of the polymer backbone. Above a threshold temperature, the changes are irreversible, leading to eventual thermal degradation of the monolayer.

11:00am **SS2+NS+BI+EL-TuM9 An Estimation of Effective Mean Free Path of Photo- and Auger Electrons in Partial Yield Measurements using Self-assembled Monolayers**, ***M. Zharnikov***, *S. Frey, K. Heister, M. Grunze,* Universität Heidelberg, Germany

In the partial electron yield (PEY) acquisition mode commonly used in X-ray absorption spectroscopy both elastically and inelastically scattered electrons contribute to the signal with the latter contribution presumably dominating. In this case a majority of inelastic scattering events will not result in the signal attenuation as it happens in the X-ray photoelectron spectroscopy (XPS). The scattered electrons will still have a kinetic energy in the acquisition range of the spectrometer. The related values of mean free path (MFP) should be, therefore, noticeably larger than the well-known inelastic mean free paths for electrons of definite kinetic energy. We have performed XPS and near edge X-ray absorption fine structure (NEXAFS) spectroscopy measurements for series of self-assembled monolayers of alkanethiols on gold substrate. The length of the alkyl chain and, subsequently, the film thickness was varied. In agreement with the expectations, the obtained effective MFPs for the Au 4f photoelectrons and C@sub KLL@ Auger electrons in the PEY acquisition mode exceed the respective values for the elastically scattered electrons of the same kinetic energies (the Au 4f and C@sub KLL@ electrons made up the elastic component of the acquired PEY signals). Furthermore, the PEY-MFP for the C@sub KLL@ Auger electrons increased with decreasing retarding voltage of the PEY detector, which correlates with the increasing contribution of the inelastically scattered electrons in the acquired signal. The obtained results are of importance for the analysis of NEXAFS spectra in both self-assembled monolayers and polymers. This work has been supported by the German Bundesministerium fuer Bildung, Wissenschaft und Technologie through grant No. 05 SF8VHA 1 and by the Fonds der Chemischen Industrie.

11:20am **SS2+NS+BI+EL-TuM10 Growth Process and Thermal Stability of Semifluorinated Alkanethiol Self-Assembled Monolayers on Au(111)**, ***M. Hara,*** Frontier Research System, RIKEN, Japan; *A. Suzuki,* Tokyo Institute of Technology, Japan; *K. Tamada,* National Institute of Materials and Chemistry, Japan; *H. Fukushima,* Seiko Epson Co., Japan; *T.R. Lee,* University of Houston

Growth process and thermal stability of semifluorinated alkanethiol (CF@sub 3@(CF@sub 2@)@sub m@(CH@sub 2@)@sub n@SH) self-assembled monolayers (SAMs) on Au(111) have been investigated by thermal desorption spectroscopy (TDS) and scanning tunneling microscopy (STM). The growth kinetics showed nearly the Langmuir adsorption isotherm and the etch pits were formed in the upright phase, while the striped phases were not observed in the initial growth stage for shorter (CH@sub 2@)@sub n@ semifluorinated SAMs. In TDS, no significant peaks can be obtained for dimer molecules and decomposed species, suggesting no associative desorption nor dimerization and thermal stability of the semifluorinated molecules during heating up to 650 K. Since longer (CH@sub 2@)@sub n@ semifluorinated SAMs remained the same chemisorbed state in the monolayer after annealing at around 480 K, it has been confirmed that also the alkyl chain part plays an important role for the thermal stability and the ordering in the semifluorinated alkanethiol SAMs. Following those results, we propose more detailed surface phase transition model of semifluorinated alkanethiol SAMs in the growth and annealing processes.

11:40am **SS2+NS+BI+EL-TuM11 Multi-technique Study of Self-Assembled AuCN Monolayers on Au(111) Formed by Electrochemical Deposition**, ***T. Yamada,*** Waseda University, Japan; *R. Sekine,* Shizuoka University, Japan; *T. Sawaguchi,* AIST/MITI, Japan

Two kinds of monolayers of AuCN electrodeposited on Au(111), indexed (1.15x@sr@3R-30°) and (1.41x2@sr@3R-30°), have been investigated by XPS, UPS and HREELS as well as LEED, AES and STM to determine the geometrical, electronic and vibrational properties. Electrodeposition was performed in an aqueous 1 mM KAu(CN)@sub 2@ solution by applying an electrode potential about 0 - +0.1 V vs SCE on the Au(111) crystal. Sharp LEED patterns were obtained for these two kinds of adlayers. AES indicated that both of these adlayers were composed Au, C and N without impurity. Well ordered adlattices composing domain structures (domain size ca. 10 nm) were observed by STM. XPS yielded Au 4f signals from AuCN indicating small fractional positive charges on the Au atom incorporated in AuCN. The UPS of AuCN/Au(111) was composed of the Au orbitals and weak signals from CN orbitals, assigned by relativistic DV-Xa molecular orbital calculation. The binding energies of CN orbitals are in the order of 4@sigma@ > 5@sigma@ > 1@pi@, which indicates that the C-Au bond is essentially covalent. HREELS yielded vibrational spectra similar to that obtained for AuCN crystalline powder.@footnote 1@ The C-N stretching frequencies were found to be 2140-2160 cm@super -1@, which are consistent with the covalent nature of the C-Au bond. In the frequency region below 300 cm@super -1@, loss peaks related to the Au-N bonds were seen. The (1.15x@sr@3R-30°) adlayer is concluded to be composed of -AuCN- linear chains (polymer chains) that are identical to those embedded in the AuCN crystal. For the (1.41x2@sr@3R-30°) adlayer, HREELS indicated distortion or breaking of Au-N bonds. Some structural models are proposed for this. These results reveal a special inorganic polymeric feature of the self-assembled AuCN adlayers lying parallel along the surface. @FootnoteText@ @footnote 1@G. A. Bowmaker, B. J. Kennedy and J. C. Reid, Inorg. Chem. 37, 3968 (1998).

Biomaterial Interfaces

Room 202 - Session BI+EL-TuA

Cell-Surface Interactions

Moderator: D.W. Grainger, Colorado State University

2:00pm **BI+EL-TuA1 Model Surfaces for Studying and Controlling the Adhesion of Cells**, ***M. Mrksich,*** The University of Chicago **INVITED**

This presentation will give an overview of the use of self-assembled monolayers of alkanethiolates on gold as model substrates for studying and controlling the interactions of cells with non-natural materials. This surface chemistry approach begins with monolayers terminated in short oligomers of the ethylene glycol group, because these films are inert to the non-specific adsorption of protein. Monolayers patterned into regions presenting glycol groups with the complementary regions presenting hydrophobic surfaces are excellent substrates for patterning the attachment of cells. The immobilization of ligands to these inert films gives substrates to which proteins can selectively bind, but which otherwise rule out non-specific interactions of proteins. This approach can be extended to give substrates that mediate the attachment of mammalian cells. Monolayers presenting the peptide Arg-Gly-Asp (a ligand for cell-surface integrin receptors) mediate the selective attachment and spreading of fibroblast cells. This presentation will also discuss the design of dynamic substrates that can alter, in real time, the presentation of ligands to an attached cell and hence influence the behaviors of adherent cells. These active substrates are based on electroactive monolayers that present redox-active groups which can be switched by applying electrical potentials to the underlying gold. A first example uses substrates that can be switched to turn on the immobilization of ligands. This strategy has been used to switch regions of the substrate from an inert state to a state that permits the adhesion and migration of cells. A second example uses substrates that can selectively release immobilized ligands from the monolayer. These examples establish that self-assembled monolayers of alkanethiolates on gold are an excellent model system for controlling the adhesion of cells and will find wide use both in fundamental studies for biology and in applied targets for biotechnology.

2:40pm **BI+EL-TuA3 Cell Respone to Chemically and Topographically Modified Surfaces**, ***D.S. Sutherland,*** *A.S. Andersson, K. Glasmastar, S. Petronis,* Chalmers University of Technology, Sweden*; F. Backhed, A. Richter-Dahlfors,* Karolinska Institute, Sweden*; U. Lidberg,* University of Gothenburg, Sweden*; B. Kasemo,* Chalmers University of Technology, Sweden

The properties of surfaces have long been known to influence cellular behaviour. Both the chemistry and topography of surfaces have been shown to effect different aspects of cellular response. With the advent of micro and nanofabrication it is now possibl e to study these interaction in a more detailed fashion, isolating specific surface structures and systematically varying their size and shape. In a parallel multicentre project a range of micro and nanofabricated surfaces are used in cell culture experi ments with a range of cell types. The specific surface designs were selected to give chemical and topographic cues on a range of length scales from the micron and submicron to the nanometre and were used as a set, to screen for the influence of surface st ructure on cellular behaviour. Similar sets of well-characterised surfaces were used in a number of different cell culture systems, including epithelial, endothelial, mammary gland and pancreatic cells, to look for both cell-specific interactions and g ene ric correlations. The studies have taken advantage of recent advances in microbiological techniques, focussing on different aspects of gene expression, cell differentiation and cell-cell signalling as well as more traditional adhesion, proliferation and m orphologic analysis. Examples of preliminary results obtained so far include: 1. Non-adherence/proliferation of three cell types to lipid bilayers (so called supported membranes) 2. Expression of a specific cytokine by epithelial cells is influenced by the microtopography of the surface. Additional results from ongoing studies are expected within a few months.

3:00pm **BI+EL-TuA4 Directing Endothelial Cell Attachment and Growth Using a Novel Ozone Patterning Technique**, *S.R. Webb,* ***T. Boland,*** Clemson University

Being able to modify surfaces to control cellular behavior, i.e. adhesion, spreading, migration, and or proliferation is extremely important in the development of materials for tissue engineering applications. Of particular interest in the field of vascular research are surfaces that will direct cell attachment and growth in the presence of RGD containing serum proteins, which may adsorb to the material surface. In this study, cell response to patterned materials was examined by employing highly organized monolayers of self-assembled (SAM) octadecytrichlorosilane (OTS) on silicon oxide wafers. OTS surfaces were exposed to ozone for a varying amount of time ranging from 1-4 minutes. The remaining surfaces were exposed to ozone via a micron size mask, allowing only the exposed areas to be etched. The surfaces were analyzed by ellipsometry and electron spectroscopy for chemical analysis (ESCA). Bovine aortic endothelial cells (BAEC): were cultured in MEM + 10% Fetal Bovine Serum + 1% antibiotic solution. Cells were seeded and cultured in 96 well plates in the presence of pure and patterned OTS surfaces. Cell attachment and growth of endothelial cells on pure OTS monolayers was very poor, most likely because of the denaturing of serum proteins near the surfaces. The surfaces exposed to ozone showed varying film thickness depending on the dose, and a strong carbonyl peak in the ESCA spectra, indicating the presence of an oxidized thin organic film. Cell attachment to etched surfaces and growth exceeded the control tissue culture polystyrene. Cell density increased in regions of the pattern to a confluent layer. The cell spreading and attachment on the micro-patterned surfaces suggests that the cells may be able to attach more firmly to the extracellular proteins on the patterned surfaces. The result from this cell growth study will aid in designing micro-patterned surfaces varies areas, such as, cell-based biosensors, biocomputers, and new biomaterials.

3:20pm **BI+EL-TuA5 Cellular Interactions with Self-assembled Monolayers**, ***G.J. Leggett,*** University of Manchester Institute of Science and Technology, UK **INVITED**

The development of a detailed understanding of the influence of surface chemical structure on mammalian cell attachment has been confronted with difficulties. Not only are the biological problems inherently complex, but until recently there have not been adequately well defined model surfaces for fundamental studies. The advent of self-assembled monolayers (SAMs) has promised to transform this situation, by providing well-defined surfaces with structures and chemistries that may readily be controlled, and the past five years have seen growing interest in the use of SAMs to model cellular interactions with artificial substrata. In the present work, SAMs with a range of alkyl chain lengths and terminal groups have been used in studies of the attachment of murine 3T3 fibroblasts and primary human osteoblast-like cells. The sensitivity of cellular attachment to subtle changes in adsorbate molecular structure and order has been explored. The responses of cells to micropatterned substrata formed using photopatterning methods have been explored. The organisation of structural elements, including filamentous actin organisation and focal contact formation, within the cell cytoskeleton has been explored using immunochemical methods. The effect of protein adsorption has been probed by comparing attachment from serum-free and full media, and by pre-exposing surfaces to protein solutions. Valuable insights have been gathered into the relationship between surface chemical structure and cellular behaviour.

4:00pm **BI+EL-TuA7 Artificial Networks of Rat Hippocampal Neurons on Microelectrode Arrays**, ***C.D. James,*** *A.J. Spence,* Cornell University*; N. Dowell,* Wadsworth Center/Department of Health*; H.G. Craighead, M.S. Isaacson,* Cornell University*; J. Turner, W. Shain,* Wadsworth Center/Department of Health

The construction of artificial neuronal networks from dissociated primary neurons will permit study of synaptogenesis, synaptic plasticity, and neuronal processing. However, a thorough investigation of these processes requires two important components: a flexible method of producing patterned cell networks, and long-term (weeks) studies of such cell networks. To address these issues, microelectrode arrays have been fabricated to conduct long-term, non-invasive extracellular measurements of spontaneous and induced action potentials. In addition, we have used two methods, microcontact printing and conventional photolithography, to align patterns of molecules, such as poly-L-lysine and laminin, to the microelectrode arrays. Surface analysis of the patterned molecules was completed to assess the relevant factors for successfully promoting cell attachment and neurite guidance. Issues dealing with the reliability and stability of the microfabricated electrode arrays, specifically for primary neuron cell cultures, will also be addressed.

4:20pm **BI+EL-TuA8 Living Neural Cells as Components in Sensors and Computational Devices**, ***J.J. Hickman,*** Clemson University **INVITED**

We are developing the methodology to build hybrid biological/non-biological systems to create new information technology devices. This presentation will focus, from a bioengineering standpoint, the steps necessary to build such a device and some of the possible functions of

these devices. We are using self-assembled monolayers (SAMs) to control the intrinsic and geometric properties of surfaces in contact with biological systems. The use of surface modification techniques allows us to tailor the interface between biological/nonbiological materials independent of the bulk composition of the nonbiological material. The ability to control the surface composition of the in vitro system as well as controlling other variables, such as growth media and cell preparation, all play important roles in creating a defined system for devise operation. This defined system has been used as a test-bed to evaluate surface coatings for neuronal interactions with electronic materials. We have used the geometric control of the surface composition afforded us by SAMs to create in vitro circuits of mammalian neurons. We have also recorded the electrophysiological signals produced by neurons on the patterned SAMs in response to stimuli. The surfaces have been characterized by X-ray photoelectron spectroscopy (XPS), imaging XPS and contact angle measurements and we have related the intrinsic properties of the surface and the proteins deposited by the cells to cellular development. We are using what we learn for a more fundamental understanding of cellular development and also to create sensors using living neurons as the sensor element. The continuing development of this technology will be discussed, our latest results, as well as the implications and applications for (a) biosensor fabrication, (b) neuronal circuit design, and (c) biological computation.

5:00pm **BI+EL-TuA10 Tissue Formation of Hepatocytes on Micro-Porous Films of Polylactide**, ***T. Nishikawa***, RIKEN, Japan; *K. Nishikawa, R. Ookura, J. Nishida, S.-I. Nishimura, H. Ookubo, H. Kamachi, M. Matsushita, S. Todo,* Hokkaido University, Japan; *M. Shimomura,* RIKEN, Japan

Control of interaction between cells and material surfaces has been considered as a fundamental issue in designing and developing bio-materials for various purposes such as cell culture, implantation, and tissue regeneration. Surface morphology is one of the factors which can control the interaction. We previously reported that two-dimensional regular honeycomb pattern appear as a surface morphology of polymer films which were fabricated by casting dilute solution of amphiphilic polymers on solid substrates in a humid atmosphere. Recently we found that the honeycomb morphology can be applied to micro-patterning of cell culture substrates and that rat hepatocytes recognize the micro-patterned surfaces from chemical and morphological aspects and change their morphology and functions. Here we show that self-supported honeycomb films can be fabricated by casting a dilute solution containing polylactide (PLLA) as major component of the films and amphiphilic polymer as component for induction of honeycomb morphology. The honeycomb films worked as cell culture substrates for rat hepatocytes. Hepatocytes on the honeycomb films formed a colony, which exhibited tissue-like structure and express high level of albumin secretion, which was comparable to that of spheroids of hepatocytes. The tissue formation of hepatocytes specifically occurred on the honeycomb films of PLLA, but not on flat films of PLLA. The colony of hepatocytes kept the morphological features and liver specific function at day 14. This indicates that micro-porous films of PLLA would be appropriate for long term culturing of hepatocytes. Recently we succeeded in culturing hepatocytes on both sides of the self-supported honeycomb films of PLLA. In this sense, we believe that our materials possessing regular micro-pores are applicable to artificial extra-cellular matrices for tissue engineering.

Processing at the Nanoscale/NANO 6
Room 302 - Session NS+NANO6+SS+MC-TuA

Self-assembly and Self-organization

Moderator: R. Wiesendanger, University of Hamburg, Germany

2:00pm **NS+NANO6+SS+MC-TuA1 Dip-Pen Nanolithography: A New Tool for Generating and Studying Soft Nanostructures**, ***C.A. Mirkin***, *S.A. Brennan, L.M. Demers, S. Hong, P.V. Schwartz, D.A. Weinberger,* Northwestern University **INVITED**

A new type of ultrahigh resolution soft-lithography, Dip-Pen Nanolithography (DPN) that is interfaceable with biomolecules and biofunctionalized building blocks will be presented. This soft lithography allows one to routinely pattern structures, in serial or parallel fashion, with sub 50 nm spatial and line-width resolution with near-perfect alignment. Implications in materials synthesis, electronics, and biodiagnostics will be discussed.

2:40pm **NS+NANO6+SS+MC-TuA3 A Step Toward Making and Wiring-up Molecular-Scale Devices with a Self-Directed Growth Process**, *G.P. Lopinski, D.D.M. Wayner, D.J. Moffatt,* National Research Council of Canada; ***R.A. Wolkow***, National Research Council of Canada, Canada

Our understanding of and control over molecular adsorption on silicon has advanced very significantly in the last several years. It is now possible to provide a microscopic picture of structure and bonding in covalently attached molecule-silicon surface systems. This detailed understanding of adsorbate-surface structures was entirely lacking when the first wave of enthusiasm for molecular devices crested roughly 20 years ago. While many ideas for molecule-scale devices have been put forward in the past, the tools - both synthetic and analytical - to pursue those ideas did not exist. Now, the control necessary to begin exploring ways to incorporate organic function into existing technologies or, eventually, to make new molecule-scale devices is within reach.@footnote 1@ Experimental and modeling methods have emerged that effectively extend the resolution of STM to see the details of adsorbed molecule structure and bonding. In the next several years it is now realistic to expect structures and concepts dreamed about for decades to begin to be realized. This talk will focus on a self-directed growth process for creating molecular nanostructures on silicon.@footnote 2@ @FootnoteText@ @footnote 1@ Controlled Molecular Adsorption on Si: Laying a Foundation for Molecular Devices, R.A. Wolkow, Annual Review of Physical Chemistry, volume 50, 413-41, 1999. @footnote 2@ Self-Directed Growth of Molecular Nano-Structures on Silicon, G.P Lopinski, D.D.M. Wayner and R.A. Wolkow, Nature in press.

3:00pm **NS+NANO6+SS+MC-TuA4 Control of Spatial Distribution of Self-Assembled Diacetylene Compounds by Co-deposition with Fatty Acid Molecules**, ***Y. Kuwahara***, *G.-M. Zhang, J.-W. Wu, M. Akai-Kasaya, A. Saito, M. Aono,* Osaka University, Japan

Control of self-assembled surface structure of functional organic molecules has been attracting intensive interest from a viewpoint of future applications such as novel material structures for nanometer-scale molecular devices. We have investigated self-assembled surface structures of two different chain organic molecules co-adsorbed on HOPG by use of scanning tunneling microscopy. The subject molecule was 10,12-tricosadiynoic acid, which is one of the diacetylene compounds possessing the possibility of being polymerized into macromolecular wire and/or sheet, and several kinds of fatty acids were used as buffer molecules. We used Langmuir Blodgett method for the fabrication of the molecular monolayers. In order to achieve the parallel molecular arrangement, the surface pressure for the deposition was deliberately controlled much lower than the saturate pressure and the substrate was horizontally oriented. A variety of molecular patterns inside the two-component monolayers were revealed, which could be briefly grouped into 'phase separation pattern', where microscopically pure 10,12-tricosadiynoic acid and fatty acid were observable respectively, and 'alternative pattern', in which the lamellae of the two sorts of molecules emerged alternatively. In order to evaluate the mechanism for the two dimensional surface ordering, we have also done the ab-initio molecular orbital calculation and the proposed structural model of the surface self-assembly is in good agreement with the theoretical simulations. Consequently, the possibility of controlling the spatial distribution of the diacetylene compounds on the solid surface has been demonstrated.

3:20pm **NS+NANO6+SS+MC-TuA5 The Interaction of Metal Atoms with Self-assembled Organic Monolayers**, ***A.V. Walker***, *B.C. Haynie, N. Winograd,* The Pennsylvania State University

Organic monolayers show great promise as materials for a wide range of technological applications. An understanding of the nature of the metal atom - organic monolayer interaction is vital in the development of molecular electronic devices. Recently it was demonstrated that deposited Al atoms can penetrate through an n-alkyl monolayer to the monolayer / Au (111) interface. This phenomenon is believed to occur via thermally activated transient defects in the monolayer. In this paper, we explore the thermodynamics of this system using time-of-flight secondary-ion-mass-spectrometry (TOF SIMS) and demonstrate that at low temperatures the rate Al atom penetration into the monolayer is reduced. We have also studied the interaction between other promising molecular wire candidates and metal atoms.

3:40pm **NS+NANO6+SS+MC-TuA6 Chiral Surface Reconstruction by Largish Molecules**, ***M. Schunack***, *L. Petersen, A. Kühnle, E. Laegsgaard, I. Steensgard, F. Besenbacher,* University of Aarhus, Denmark

Temperature-controlled scanning tunneling microscopy studies provide insight into the bonding, ordering and mobility of large organic molecules

at metal surfaces. This is illustrated by investigations of disc-like molecules on a Cu(110) surface with a variable temperature STM, which can be operated down to 25 K.@footnote 1@ Hexa-tert-butyl decacyclene (HtBDC) self-assembles upon deposition onto Cu(110) above 250 K and forms a double-row structure in two directions surrounded by fast-diffusing single molecules. Nano-manipulation experiments with the STM at low temperatures revealed an underlying chiral reconstruction of the Cu surface. This consists of holes of approximately 14 Cu atoms pulled out of the surface. Surprisingly, the observed reconstruction is chiral. By gently annealing of the molecule structure at higher coverages, large enantiomerical pure domains with two different orientations build up. By means of simple effective medium theory calculations, we estimate the lower bound of the adsorption energy to be E@sub ad@ = 0.45 eV, and can give a plausible explanation for the observed structure. @FootnoteText@ @footnote 1@ L. Petersen, M. Schunack et al., submitted to Review of Scientific Instruments.

4:00pm **NS+NANO6+SS+MC-TuA7 From Functionalisation of Single Molecules to Self-organisation of Nano-structured Thin Films**, ***Q. Guo***, University of Birmingham, U.K. **INVITED**

The combination of functionalisation of individual molecules with self-organisation of the molecules into functional structures is a promising route for the fabrication of nanoscale electronic and optoelectronic devices. In this talk I will present experimental findings of nanostructured thin films prepared using this method. The dependence of the structure of molecular monolayers on the functionality of individual molecules will be demonstrated using chemisorbed acetate and benzoate species on TiO@sub 2@ surfaces as an example. Both acetate and benzoate attach to the substrate through strong bonding between the carboxyl end of the molecules and the metal cations at the surface of TiO@sub 2@. In the case of benzoate, the phenyl ring offers an extra functionality for intermolecular linkage, leading to the formation of dimerised rows of benzoate. Self assembled monolayers (SAMs) of functionalised alkanethiols adsorbed on Au(111) surfaces will also be discussed. Functionalisation of the tail group of thiol molecules gives rise to SAMs with different surface energies, allowing the fine tuning of the reactivity of the surfaces towards binding of deposited atoms and molecules. The formation of nano-particles of gold on carboxyl terminated SAMs has been investigated using scanning tunnelling microscopy (STM) and electron energy loss spectroscopy(EELS) and results will be presented to demonstrate the important role of functionality of individual molecules on nano-structure formation.

4:40pm **NS+NANO6+SS+MC-TuA9 Ion Beam Assisted Self-Organization of Periodic Nanowire-Arrays on CaF2 Substrates**, ***M. Batzill***[1], *F. Bardou, K.J. Snowdon,* University of Newcastle, UK

The fabrication of well ordered nanowire arrays over large areas is a challenge with many potential applications. Here we report a novel glancing incidence ion beam assisted self-organisation approach to form periodic wire-arrays over large areas on a CaF2(111) substrate. Preferential erosion of fluorine by the ion beam creates a surface enriched in calcium. The calcium self-organises in elongated island structures of preferential width and separation. If the sample is irradiated along a fixed azimuth we observe formation of nanowires with ~10 nm periodicity and wire lengths of at least several micrometers oriented along the azimuthal direction of ion beam incidence. Electrical conductivity measurements reveal a three order of magnitude lower conductivity normal to the wires than along the wires.

5:00pm **NS+NANO6+SS+MC-TuA10 Quantum Engineering of a Pb Nanostructure: Controlling the Thickness with Monolayer Precision**, ***C.-S. Jiang***, *H.-B. Yu, X.-D. Wang, C.-K. Shih,* University of Texas at Austin

We report a novel quantum engineering of Pb mesas on Si(111), designing the quantum number (N) of the electron resonator of Pb by modifying its thickness with monolayer precision. Pb deposition on Si(111) forms mesas on the surface, and the mesas serve as electron resonators because of the strong quantization along the surface normal direction. To modify the mesas into desired thickness, mass-transfer was first triggered by an STM-tip under controlled conditions. The triggering lead to the formation of single layer with annular shape at the edge of the mesa by transferring the Pb mass from the wetting layer. Once triggered, the mass transfer from the wetting layer to the top of the mesa continue until the new layer involving millions of atoms is completed. Once this layer is completed, no more mass transfer is observed unless a new triggering is performed. Each triggering leads to addition of one complete monolayer on top of the plateau. Using this process, the Pb mesa thickness can be engineered in a quantized fashion. Detailed mechanisms involved in the engineering will be discussed.

[1] **NSTD Student Award Finalist**

Biomaterial Interfaces
Room 202 - Session BI+SS-WeM

Water at Biointerfaces

Moderator: B. Kasemo, Chalmers University of Technology, Sweden

8:20am **BI+SS-WeM1 Role of Water in Biological Processes**, ***E.A. Vogler,*** The Pennsylvania State University **INVITED**

Water is so familiar and ubiquitous in our environment that we frequently forget, ignore, or underestimate the role its special properties play in the biological and material sciences. Likewise in the hybrid field of biomaterials, where water-surface interactions apparently control and/or moderate the biological response to materials applied in medicine and biotechnology. In fact, the behavior of water near surfaces is becoming increasingly relevant in these fields as surface-to-volume ratios increase with the ever-decreasing size imposed by micro-to-nano-scale analytical and medical devices, especially as applied in nanobiotechnology. This lecture reviews some important water properties from both a thermodynamic and molecular perspective. The occasionally forgotten biological/environmental importance of thermodynamic attributes such as heat capacity, (latent) heats of fusion/vaporization, density, and interfacial tension are briefly recapitulated, leading to the conclusion that terrestrial life simply could not and would not work the way it does without the special mediating influence of water. Molecular aspects that give rise to these important bulk-water properties will be discussed along with the impact these have on the mechanisms of water wetting. It will be suggested that current theories of wetting substantially ignore some of these unique aspects of molecular water that distinguish it from all other room-temperature fluids. Against this backdrop, evidence suggesting that the role of water in the acute biological response to materials has been underestimated will be presented, concluding that the interfacial behavior of water must be explicitly included in any comprehensive biophysical theory attempting to explain or predict performance of biology at aqueous interfaces.

9:00am **BI+SS-WeM3 Neutron Reflectivity Studies on the Interaction of Water with Biocompatible Monolayer Films**, ***D. Schwendel,*** University of Heidelberg, Germany*; R. Steitz,* Hahn-Meitner Institute, Germany*; J. Pipper, R. Dahint, M. Grunze,* University of Heidelberg, Germany

Since the early 1990s protein resistance has been obtained for surfaces coated with poly- or oligo(ethylene glycol) (PEG or OEG) derivatives ((CH@sub2@-CH@sub2@-O)@subn@). While the inertness of PEG has been explained by the steric repulsion theory associating the inertness of the polymer brushes with the high conformational freedom of PEG chains in the near surface region, FT-IRRAS studies related the protein resistance of OEG to its molecular conformation. Whereas the helical and amorphous conformers on Au are inert towards protein adsorption, the planar all-trans conformer on Ag does adsorb protein. In Small Angle Neutron Scattering (SANS) studies we found experimental evidence for a strongly bound water layer on helical OEG-terminated alkanethiolate self assembling monolayers (SAMs). The experimental observations have been predicted by ab initio calculations simulating the adsorption of water molecules on methoxy terminated OEG with 3 EG units (EG3-OMe) and Monte Carlo simulations of water close to the SAM surfaces. Both theoretical studies postulate easy accommodation of water at helical OEG strands and a lower density of water near the SAM surface. This strongly bound water film is identified as the physical cause that these surfaces are inert against protein adsorption and cell attachment. SANS studies showed that the data for a hydroxy terminated helical OEG-SAM with 6 EG units (EG6-OH) on Au correlate satisfactorily with the model assuming a boundary water layer of 56 Å at the solid/liquid interface with a density of 92 % of that of bulk water. Also for the investigation of amorphous EG3-OMe immobilized on Au the assumption of a 36 Å water layer with a density of 78 % compared to bulk D@sub2@O yielded a much lower @chi@@super2@ deviation between the experimental data and the fit than the assumption of no interphase water.

9:20am **BI+SS-WeM4 Hydrogen Bond of Water in Ih Ice Probed by Core-level Spectroscopies**, ***H. Ogasawara,*** *D. Nordlund, M. Cavalleri, L.-A. Näslund, M. Nagasono, L.G.M. Petterson, A. Nilsson,* Uppsala University, Sweden

In biological complexes, DNA, protein and so on, materials consist of two types of chemical bonds. A shorter covalent bond has the strength of a few eV to construct molecular flame, and longer hydrogen bond has the strength of a few tenth meV. This energetically weak nature of hydrogen bond gives flexibility and enables the self-organization of molecules at ambient temperature. Ice is a unique material that hydrogen and oxygen atoms in the crystal are connected both covalent and hydrogen bonds where each oxygen atom has two covalent O-H bonds and two hydrogen O-H bonds. Here we report electronic structure of water in Ih ice, a thin film grown on Pt(111), studied with combination of core-level spectroscopies, X-ray absorption spectrosocpy (XAS), X-ray emission spectroscopy (XES) and theoretical simulation (DFT calculation). The experiments were performed at MAX-LAB, Sweden. The details of the endstation is described elsewhere.@footnote 1@ The theoretical simulation of spectra was done using the deMon program@footnote 2@ XAS and XES results indicate a reconfigration of molecular orbitals of water in ice beside the binding energy shift. In O 1s XAS, 4a@sub 1@ resonance is severely suppressed indicating the enhancement of s-character of this orbital compared to that of the gas phase. In O 1s XES, 3a@sub 1@ emission is suppressed showing the strong s-character of this orbital. From these observations we conclude that a water molecule in ice has a pseudo-totally-symmetric character. This pseudo-totally-symmetric character of water in ice is confirmed by excitation profile of 3a@sub 1@ photoemission peak and theoretical simulation. @FootnoteText@ @footnote 1@ R. Denechke et al, J. Electron Spectrosc. Relat. Phenom. 101-103, 971(1999). @footnote 2@ deMon-KS version 4.0, deMon Software, (1997).

9:40am **BI+SS-WeM5 Tyrosine Derivatives Adsorbed on Gold for Surface Modification**, ***K. Uvdal,*** *J. Svensson, P. Konradsson, B. Liedberg,* Linköping University, Sweden

Model molecules can be very useful when searching for mechanisms of protein folding. We intend to use model molecules to study if the changed conditions for binding of water, caused by binding and cleavage of ATP, is the main reason for protein conformational changes. In this very first study we are investigating tyrosine derivatives linked to 3-mercaptopropionic acid through an amide bond. Two different tyrosine derivatives, one with the OH group free and one with the OH group phosphorylated are studied. These molecules are adsorbed on gold and studied by X-ray Photoelectron Spectroscopy (XPS), Infrared Reflection-Absorption Spectroscopy (IRAS). The techniques are used to investigate the coordination to the surface and the molecular orientation of adsorbates relative to the surface. Molecular surface interactions causing chemical shifts in the core level XPS spectra of the adsorbates on gold are investigated using multilayer films as references. IR in transmission mode is used as a reference to the IRAS results and thus the surface selection rule is used to identify the orientation of certain vibrations relative to the surface. The S(2p) core level XPS spectrum for the adsorbate of the tyrosine derivative shows only one type of sulfur. The S(2p3/2) peak is shifted about 2.5 eV to lower binding energy when compared to multilayer showing a chemical adsorption through the sulfur atom. A phosphorylated tyrosine derivative adsorbed on gold shows an enhanced signal from PO3 in surface sensitive mode in good agreement with a molecular orientation with the PO3 group pointing away from the surface. The IR spectrum of the tyrosine derivative is showing several strong bands in transmission mode (KBr). Most of these peaks are also strong in the spectrum for the adsorbate. However, some significant differences are observed which are correlated to the molecular orientation relative to the surface. In a second step these monolayers are to be used for water interaction studies.

10:00am **BI+SS-WeM6 Stretching of a Macromolecule: A First Principles Theory**, ***H.J. Kreuzer,*** Dalhousie University, Canada **INVITED**

The statistical mechanics to describe the stretching of a single polymer strand (in particular in an AFM experiment) is formulated. As ingredients one needs the potential energy surfaces of the various conformers of the macromolecule. These have been calculated for oligo (ethylene glycol) resulting in quantitative agreement for the force/extension curves measured for PEG, both in hexadecane and in water. The interaction of water with PEG is discussed in great detail. We also present results for the effect of strong electric fields on PEG, such as surprisingly large electrostriction.

10:40am **BI+SS-WeM8 A Fundamental Approach to Protein Adsorption: Changes in Free Energy for Adsorption of Individual Peptidyl Residues onto Functionalized SAM Surfaces**, ***R.A. Latour,*** Clemson University*; L.L. Hench,* Imperial College, UK

Cellular response to biomaterial surfaces has great importance for the design of bioactive substrates for implant, drug delivery, and tissue engineering applications and is greatly influenced by protein/surface adsorption. All proteins are made up of amino acids (peptidyl residues); thus protein adsorption must be fundamentally governed by the submolecular interactions between a protein's residues and surface

functional groups. The objective of this research was to develop an approach to quantitatively determine changes in Gibbs free energy for individual mid-chain residue/surface (R/S) functional group interactions and apply it to selected R/S group pairs. Molecular models (MOPAC/COSMO; CAChe software, Oxford Molecular Inc.) were created of 3 residues (Ala, Ser, Lys) and 3 SAM surfaces (CH@sub 3@, OH, COO-) to represent hydrophobic, hydrophilic, and charged systems in an aqueous environment. Adsorption enthalpy for each R/S pair was determined by positioning the residues over the surfaces and calculating the system energy as they were sequentially separated from the surface. Additional enthalpy and entropy contributions due to water restructuring effects were estimated based on changes in solvent accessible surface area and experimental wetting data. This was combined with the modeling data to calculate the net @DELTA@G@sub ads@. Ala was predicted to tightly bind to the CH@sub 3@ surface with @DELTA@G@sub ads@ = -5.8 kcal/mol with Ser and Lys each having @DELTA@G@sub ads@ > 0. All 3 residues exhibited @DELTA@G@sub ads@ > 0 for adsorption to the OH surface. Lys was predicted to be attracted to the COO- surface with @DELTA@G@sub ads@ = -5.4 kcal/mol, but only through intervening water layers with a 5 - 7 Å surface separation distance. Ala and Ser had @DELTA@G@sub ads@ > 0 for their interaction with the COO- surface. Further work is planned to integrate this type of data to develop a universal model for predicting protein-surface adsorption behavior.

Biomaterial Interfaces
Room Exhibit Hall C & D - Session BI-WeP
Poster Session

BI-WeP1 Hydroxy-containing Amino Acid Derivatives Adsorbed on Gold, *J. Svensson,* ***A. Borgh****, K. Uvdal, B. Liedberg, P. Konradsson,* Linköping University, Sweden

More than 10% of the proteins in the body are involved in phosphorylations. As a phosphate group is attached to serine, threonine or tyrosine, the protein changes its conformation. This change in conformation is often ascribed to neutralization of charges, i.e. by electrostatic interactions. The attachment also effects the surrounding water structure and this change in water structure could be the reason to conformational changes. We describe here the preparation of a series of model surfaces, based on self-assembly on gold, in order to study the surrounding water structure. Surface characterization is performed using infrared reflection-absorption spectroscopy (IRAS), ellipsometry, contact angle goniometry and X-ray photoelectron spectroscopy. The molecules are found to form highly ordered monolayers and the thicknesses of the monolayers are in good agreement with expected values from space filling models. Water structure studies are performed using IRAS and temperature programmed desorption.

BI-WeP2 Template Stripped Gold Surfaces for Advanced Biological Applications, ***M. Hasselblatt****, F. Zaugg, P. Wagner,* Zyomyx, Inc.

Template Stripped Gold (TSG) surfaces have been used extensively as a source for ultra-flat substrates. Thin Gold films evaporated on a freshly cleaved mica surface at elevated temperatures are glued onto a substrate to allow the removal of the mica. The gold films accessible this way exhibit sub-nanometer roughness over areas larger than micrometers. Here, we review methods for preparing these surfaces. Also, we present a novel bonding technique, in which Indium solder is used. Such sandwiches resist all common organic solvents and aqueous buffers typically used for biologically relevant experiments.

BI-WeP3 Macroporous TiO@sub 2@ Films Prepared on Ti Surfaces with Predefined, Micron-Sized Pores, ***F.A. Akin****, L. Hanley,* University of Illinois at Chicago

Surface morphology is critical to the biocompatibility of hard tissue implants, such as those used for dental and orthopedic applications. Porous bioceramics are commonly used for some applications due to their ability to encourage bone ingrowth, but they lack the physical strength required for load bearing applications. A new method is described for preparing thick, macroporous TiO@sub 2@ films on Ti surfaces with pre-defined, micron-sized pores. Scanning electron microscopy shows that these pores 1) are controlled to a narrow size range by the synthetic process, 2) can be prepared from 0.5 to ~100 µm diameter, and 3) are interconnected within the film. The films are ~0.1 mm thick and strongly adherent to the Ti surface. X-ray photoelectron spectra indicate that these films can be prepared as elementally pure TiO@sub 2@. X-ray diffraction indicates that the films are monophasic as either anatase or rutile TiO@sub 2@. Scanning electron microscopy following immersion in Ringer's solution for several days shows that the films are not expected to undergo resorption following implantation. The effect of pore size is examined for fibroblast proliferation and spreading in vitro. These macroporous TiO@sub 2@ films should allow applications that combine the favorable mechanical properties of Ti metal implants with the tissue engineering enhancements possible with porous bioceramics.

BI-WeP4 Photovoltaic Characteristics of bR/p-Silicon Heterostructures using Surface Photovoltage Spectroscopy, *L.S. Li, T. Xu, J. Jin, Y.J. Zhang,* Jilin University, P.R. China*;* ***T.J. Li****,* Jilin University, P.R. China, P.R.China*; B.S. Zou, J.P. Wang,* Georgia Institute of Technology

In bR-based photoelectric devices, the highly efficient electric response can be obtained only when the bR molecules have a nonrandom orientation. LB technique enables molecular-order organization that can be used to incorporate bR into devices. As a prototype molecular electronic device, it will be more significant to deposit oriented bR films on a silicon substrate. In this paper, orientated bR films were deposited on the hydrophilic and hydrophobic silicon substrates using the LB technique. The cytoplasmic (CP) or extracelluar (CE) surface of bR face the silicon directly, giving oriented patterns of Si/CP-EC and Si/EC-CP, respectively. The photovoltaic features and interfacial charge separation of p-Si/bR/ITO heterostructure are studies by surface photovoltage spectroscopy (SPS). The different photovoltage response values obtained are due to the nonrandom orientation of bR in the LB films on the hydrophilic versus hydrophobic silicon substrates. The photovoltage response value versus external potential of the p-Si/CP-EC/ITO heterostructure shows an obviously rectifying behavior. Compared with the p-Si/ITO heterostructure, the response value of SPS increases more rapidly in the case of the positive external potential.

BI-WeP5 Patterning Hybrid Surfaces of Proteins and Supported Lipid Bilayers, ***L.A. Kung****, L.C. Kam, J.S. Hovis, S.G. Boxer,* Stanford University

Two methods for patterning surfaces with supported lipid bilayers and immobilized protein are described. First, proteins are used to fabricate corrals for supported lipid bilayers. Poly(dimethylsiloxane) (PDMS) stamps are used to deposit arbitrarily-shaped patterns of thin layers of immobilized protein onto glass surfaces. This is followed by formation of supported lipid bilayers via vesicle fusion into the regions that are not coated with proteins. Second, supported bilayer membranes are blotted to remove patterned regions of the membrane,@footnote 1@ and the blotted regions are filled in (or caulked) with protein from solution. In both cases, the lipid bilayer regions exhibit lateral fluidity, but each region is confined or corralled by the protein. These two methods can be combined and used iteratively to create arrays with increasing lateral complexity in both the fixed protein and mobile supported membrane regions for biophysical studies or cell-based assays. @FootnoteText@ @footnote 1@ Hovis, J. S.; Boxer, S. G. Langmuir 2000, 16, 894-897.

BI-WeP6 Individually Addressable Solid Supported Membranes Formed by Micromolding in Capillaries, ***S. Kuenneke****, A. Janshoff, H. Fuchs,* WWU Muenster, Germany

The formation of spatially individually addressable, patterned biomaterial on surfaces is of paramount interest for the development of biosensors, combinatorial libraries, and high-throughput systems for pharma screening. Particularly, the combination of high resolution scanning devices with lithographically structured biomolecules is advantageous if the amount of biomaterial is limited or if the number of surface reactions is vast. The most versatile matrix for embedding and immobilizing natural and artificial receptor molecules such as functionalized lipids or proteins are solid supported membranes. Here we present a new type of microstructured membrane compartments, which are individually addressable by the operator on a common substrate on a nanometer to micrometer scale. The membrane segments are designed to be accessible to all available microscopic techniques and surface analysis tools. We developed a general procedure to generate patterned lipid bilayers by using a three dimensional network of capillaries as provided by microfluidic networks. The fluidic network (elastomer stamp) was formed from polydimethylsiloxane (PDMS) using an appropriate master displaying the inverted desired structure, which can be conveniently obtained by optical lithography of silicon wafers. Lipid bilayers were deposited by fusing unilamellar vesicles on the hydrophilic glass substrate. Visualization of the liposome flow in the capillaries and the formed planar bilayers was performed using a confocal laser scanning microscope. The planar bilayers were subsequently imaged by means of scanning force microscopy revealing a typical height of 4-6 nm.

BI-WeP7 Vesicle -> Supported Bilayer Transformation Kinetics; Influence of Support Material, Vesicle Size and Temperature, ***E. Reimhult****, F. Höök, B. Kasemo,* Chalmers University of Technology, Sweden

Supported phospholipid bilayers (SPB) on a solid surface are biologically functional components of high current interest, e.g., for biosensors, tissue engineering, and basic science (Sackman, Science 271:43 (1996); Stelzle et al., J. Phys. Chem. 97:2974 (1993)). We have recently reported the kinetics of SPB formation from sonicated, unilamellar vesicles (SUV) of average size 25 nm, on a SiO@sub 2@ support, using the quartz crystal microbalance - dissipation (QCM-D) technique (Keller and Kasemo, Biophysical Journal 75:1397 (1998); Keller et al., Phys. Rev. Lett. 84:5443 (2000)). Several interesting questions arose from the latter results; how does the vesicle -> bilayer transformation kinetics depend on the vesicle size, on temperature, and on the support. In the present study we are addressing these questions, whose answers are important for future sensor, biomaterial and micro-patterning applications. Already obtained results reveal a (vesicle) size-dependent kinetics, where also the end result (the final bilayer) may have different properties for different sizes of extruded unilamellar vesicles (EUV). The dependence on temperature is currently studied and will be reported. An exploratory study shows a strong temperature dependence for the vesicle -> bilayer transition. Different surfaces also cause different kinetics. So far, SiO@sub 2@ surfaces have been the dominating support used to promote complete bilayer formation. Vesicle adsorption, but no

bilayer formation, is observed for oxidized Ti and Au surfaces , while partial bilayer formation may occur on Pt. The above results constitute a platform from which more complex functional supported bio-membranes can be constructed (Höök et al, to be published).

BI-WeP8 Use of Bacterial Adhesion Related and Collagen Related Peptides to Bind and Orient Fibronectin on Surfaces, ***U. Klueh***, *D.L. Kreutzer, J.D. Bryers,* University of Connecticut, Schools of Medicine and Dentistry

Although small molecular weight proteins and peptides have been bound and oriented on surfaces, little is known about orienting large molecular weight proteins, (e.g. FN) on surfaces. Recently two classes of peptides have been shown to bind to FN in vitro, i.e. Collagen Related Peptides (CRP) and Bacterial Adhesion Related Peptides (BARP). We hypothesized that if these peptides could be used to not only bind, but also orient FN on surfaces. We further hypothesized that antibodies to specific regions of the FN molecule can be used to demonstrate the orientation of the peptide bound FN. CRP and BARP peptides were synthesized commercially and immobilized on polystyrene surfaces. FN was nonspecifically bound to polystyrene by physisorbtion. FN binding to the immobilized peptides was quantitated using monomeric/functional @super125@I-FN and polyclonal antibodies to FN. Orientation of the bound FN was demonstrated using antibodies specific to the amino (anti-N) and carboxyl (anti-C) termini of FN. Polystyrene immobilized CRP and BARP bound 125 ng/cm@super2@ and 94 ng/cm@super2@ of FN. Little FN bound to control (non-peptide containing) surfaces 5 ng/cm@super2@. FN bound to CRP and BAR peptide bound anti-FN and anti-C antibodies but did not bind significant levels of anti-N antibodies, compared to randomly bound FN (i.e. physisorbed FN). Additionally, we demonstrated that although CRP did inhibit FN binding to immobilized collagen, BRAP did not. Finally we demonstrated that the uses of monomeric/functional FN was critical in establishing FN monolayers on CRP or BARP coated surfaces. Our results not only demonstrate the ability of CRP and BAR peptide to specifically bind and orient fibronectin in monolayers, but also underscore the usefulness of specific polyclonal and monoclonal antibodies to characterize the binding and orientation of FN on these surfaces.

BI-WeP9 Conformational Changes of the Extracellular-matrix Protein Fibronectin Induced by Force Spectroscopy, ***Y. Oberdoerfer***, *H. Fuchs, A. Janshoff,* WWU Muenster, Germany

Since its invention, force spectroscopy by SFM became a powerful instrument to study the structure, mechanism and behaviour of polymers. Especially for biopolymers it is important to be able to perform these studies in a native environment, an advantage which is provided by SFM. In this work we studied conformational changes of the extracellular-matrix protein fibronectin and provide direct proof for the presence of the protein on the cantilever verifying whether it was pulled on the polymer or not. Fibronectin is a modular protein consisting of three different FN-domains: FN I, FN II and FN III. These three domains differ in the number of their appearance in one single fibronectin polymer and also in the number of amino-acids. Extending fibronectin during a force-measurement results in unfolding events of single domains can be distinguish in a force-curve due to the elongation of the polymer itself and also the absolute number of unfolded domains. In this way it is possible to determine whether a FN I-, FN II- or FN III-domain was unfolded. Another topic that should be presented is the possibility to make an elemental mapping of a cantilever surface by means of SIMS and SNMS to determine if the investigated polymer was attached to the probe. With this kind of measurement one can verify if unfolding events occurring in a force-curve arise from the substance itself or any kind of contamination.

BI-WeP10 Observation of Bone Fracture Healing Processes by Atomic Force Microscopy, ***V. Baranauskas***, *I.G. Freitas, Z. Jingguo, M.A. Cruz-Hofling,* Universidade Estadual de Campinas, Brazil

Atomic Force Microscopy (AFM) was used to study the healing process of bone surgical fracture in rats. We used young male adult rats (Wistar), with corporal masses between 250 and 300 grams. Each fracture was provoked by drilling a 2 mm diameter hole in one cortical tibia surface. The healing course was monitored at 8, 15 and 22 days after the fracturing. AFM images, at different magnifications, allowed to the identification of the time dependence of the osteoblastic activity, measured by the increase in the primary bone trabeculae surface and the increase in the synthesis and organization of collagen fibers of the bone matrix. Characterization of the natural recovery of the damaged bone tissue by AFM is potentially of great importance because it allows the comparison of natural recovery and the recovery induced by medicines or other cures.@footnote 1@ @FootnoteText@@footnote 1@ I. G. F. Freitas, V. Baranauskas, M. A. Cruz-Höfling, Laser effects on osteogenesis, Applied Surface Science 154-155 (2000) 548-554.

BI-WeP11 Dielectric Characterisation of Aminal Bone, ***S. Mohiuddin***, King Saud University, Saudi Arabia

The investigation deals with the electrical properties of bovine bones. Dielectric constant, dielectric loss, conductivity and resistivity are determined in bovine tissues are lossy dielectric. They are partly polarisable and partly conductive. The influence of calcium phosphate on the electrical behavior of bone is also studied. Data of electrical parameters of bovine scapula, rib and femur bones reveal the considerable variation in different bones samples and also in different specimens of the same bone, obtained from various parts of the bone. This may be attributed to the inhomogeneous deposition of calcium phosphate and water content of the bones. The sharp fractional changes in dielectric constant and resistivity with the water content of bone specimens suggests that the electrical parameters are very sensitive to free water present in bones in contrast to ultrasonic and mechanical properties. Influence of water on electrical behavior is specific to bone because of the fact that mineral content of the bone is found to be in different proportions which also effects the electrical make up of the bone. The three parameters namely water content, mineral content (calcium phosphate) and orientation of the collagen fibers with respect to the applied electric field, play an important role in influencing the electrical parameters conductivity of the bone tissue, when measured at the bulk, level.

BI-WeP12 TOF-SIMS Characterization of Nucleic Acid Biosensors, ***H.F. Arlinghaus***, *M. Ostrop, O. Friedrichs, U. Gunst,* Physikalisches Institut der Universität Münster, Germany

In recent years, biosensors consisting of immobilized oligodeoxynucleotides (ODN) have been a subject of growing interest for DNA sequencing and clinical diagnostics. We have used static TOF-SIMS and temperature-programmed SIMS (TP-SIMS) to examine in detail the immobilization process of ODNs, which were directly bound to Au- and Ag-surfaces by thyol-linkers. Protonated (M+H)@super +@ and deprotonated (M-H)@super -@ signals of the different ODNs and bases as well as phosphate ions were used to monitor the ODN concentration. The influence of ODN concentration and immobilization time on the immobilization process was investigated. It was found that the maximum intensity for characteristic ODN peaks was obtained using a 1 μM solution and an immobilization time of 24 h. According to our estimate, the surface coverage under these conditions should be close to a monolayer. Measurements of how surface structure affects the process of immobilization showed a higher intensity of the characteristic ODN signals and a less homogeneous oligonucleotide layer with increasing surface roughness. TP-SIMS was used to measure the thermal stability of the immobilized layers. The data show that the characteristic ODN fragment ions start to decrease at a temperature of about 150° C, with differences in the point of onset for the different bases. It can be concluded that the combination of TOF-SIMS and TP-SIMS is a powerful technique to examine the complexity of the immobilization and hybridization processes of nucleic acid.

BI-WeP13 Characterization of Multi-Component Adsorbed Protein Films by ToF-SIMS, ***M.S. Wagner***, *D.G. Castner,* University of Washington

Characterization of the adsorbed protein films on biomaterial surfaces is needed for the rational design of biomaterial surfaces. Many surface analysis techniques, however, do not provide efficient means for analysis of these surfaces. Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) is an excellent technique for the analysis of complex protein films due to its chemical specificity and surface sensitivity. The ToF-SIMS fragmentation pattern is highly complex due to the heterogeneity of the protein composition and the absence of unique, identifying peaks from protein to protein. Analysis of such complex spectra requires the use of multivariate analysis methods to effectively use the ToF-SIMS data. Therefore, we have utilized ToF-SIMS in conjunction with Partial Least Squares (PLS) regression to estimate the surface compositions of binary and ternary adsorbed protein films. Using only the pure component ToF-SIMS spectra, PLS was able to estimate the relative concentrations of the proteins on the surface. Furthermore, using a set of standard protein spectra, Principal Components Analysis (PCA) was able to provide insight into how the composition of a protein film adsorbed from 1% bovine plasma varied with adsorption time. PCA of the ToF-SIMS spectra revealed a shift from mostly fibrinogen to mostly immunoglobulins over the course of two hours. ToF-SIMS/PCA has the tremendous advantage that several proteins can be analyzed in a single experiment, given the appropriate

standard spectra. The combination of multivariate analysis methods and ToF-SIMS greatly simplifies the analysis of adsorbed protein films.

BI-WeP14 Correlation of Cell Health with Protein Layer Thickness on Modified Surfaces Characterized by XPS, XAS, and Morphological Analysis, ***H.E. Canavan,*** The George Washington University*; W.E. O'Grady,* Naval Research Laboratory*; J.J. Hickman, D.E. Ramaker,* The George Washington University

The interactions of biomolecules with surfaces are of significant interest in the areas of biocorrosion, bio-implant rejection, and biological fluid interactions with MEMS devices. This interaction may dictate the health of nearby cells by either a) continuing cell function as normal, b) continuing cell function in an abnormal manner, or c) cell death. In the work presented here, the biomolecular interaction is altered via prior surface modification with Self-Assembled Monolayers (SAMs) of amines or fluorinated compounds. The surfaces are then introduced into cell culture using cardiac and other cells. The interaction of the derivatized surfaces with proteins and cells are investigated to see a differential response to the modifications. X-ray Photoelectron Spectroscopy (XPS) is used as an analytical technique to characterize the modified surfaces of the silanes prior to cell culture. XPS is also used characterize the protein deposition layer thickness which serves as an indication of cell function and health. Optical microscope images of cells grown on different substrates are compared to time-dependent XPS results to correlate cell morphology with protein layer thickness. Sulfur K-Edge X-Ray Absorption Spectroscopy (XAS) data are used to monitor the extent of S-C, S-O, and S-H bonds, which affect the character of the extruded proteins.

BI-WeP15 Soft X-ray Spectromicroscopy of Protein Adsorption on Polyurethanes, ***C. Morin,*** *A.P. Hitchcock, I.N. Koprinarov, R. Cornelius, J.L. Brash,* McMaster University, Canada

New quantitative techniques for chemical microanalysis which allow detailed study of protein polymer interactions are required for biomaterial interface optimization. We are particularly interested in identification of possible preferences of first sites of protein attachment to polyurethane polymers used in blood contact medical applications. We are exploring Scanning Transmission X-Ray Microscopy (STXM) and Photoemission Electron Microscopy (PEEM) in this context. These techniques use near edge X-ray absorption spectroscopy (NEXAFS) for chemical identification. Both techniques have been used to map albumin and fibrinogen adsorbed on various polymer surfaces. As an example, STXM was used to study protein adsorption from a 0.1 mg/ml albumin solution onto a TDI-based, high-ether polyurethane film (~100 nm thick) which had submicron phase segregated regions of a highly aromatic polyisocyanate polyaddition product (PIPA) reinforcement material. Image sequences recorded throughout the C 1s and N 1s regions were used to generate composition maps by fitting the spectrum at each pixel to spectra of pure reference materials. The strong amide carbonyl resonance at 288.2 eV provides a sufficiently strong signature of protein to allow mapping down to monolayer levels even though the STXM results average over the full thickness of the polymer and protein sample. PEEM studies on similar materials provide greater surface sensitivity but are complicated by high sensitivity to topography as well as charging artefacts. Results from the two techniques will be compared to illustrate the strengths and weakness of these soft X-ray spectromicroscopy techniques when applied to biomaterials problems. X-ray microscopy is carried out at the Advanced Light Source (supported by DoE under contract DE-AC03-76SF00098), supported financially by NSERC (Canada).

BI-WeP16 A Novel Approach to Studying Structures and Orientation at the Protein-Self Assembled Monolayer Interface, ***L.F. Pardo,*** *T. Boland,* Clemson University

Self-assembled monolayers (SAMs) have become an important tool in protein adsorption studies, partly because they represent chemically, well-defined model systems. However, specifics on how the structure and orientation of both the SAM and protein change during adsorption in situ remain unknown. The purpose of this study is to quantify these changes via analysis of protein adsorption onto SAMs by a novel technique using Evanescence Reflection Spectroscopy (ERS). This technique allows in situ characterization of the surface chemistry, providing quantitative information on structure and orientation at the interface only. In this study, model proteins, (polyserine and fibrinogen) were adsorbed onto, -OH, -COOH, and CH3 terminated SAMs of hexadecanethiols on gold. The surfaces were then characterized by FTIR and ellipsomentry. The IR measurements reveal significant differences were between protein structures in dry and wet states. The spectra of polyserine in the dry state show only amide II stretch (1507 cm-1) while the amide I stretch is absent implying that the polyserine lies flat on the SAM surface. Analysis of the IR spectra measured in PBS solution depicting peaks in the Amide I and II regions showed that polyserine extends away from the surface under aqueous conditions. Furthermore, information on the secondary structure of the solvated proteins adsorbed to the various SAMs was attained. The resulting of protein-SAM interfaces are dynamic and will undergo structural changes, desorption or surface reactions. The structural characterization of protein SAM interactions will be helpful when designing templates for tissue engineering applications.

BI-WeP17 Microbial Adhesion on Polymer: Role of Morphological and Chemical Properties in the Micro-organism Behaviour, ***M. Anderle,*** *R. Canteri, E. Carli, S. Janikowska, A. Lui, C. Pederzolli, G. Speranza,* ITC-irst, Italy*; D. Maniglio, C. Della Volpe,* Università di Trento, Italy

Infections caused by implanted polymeric devices (especially catheters) have an increasing importance in the medical routine (up to 40% of nosocomial infections). The critical event in the pathogenesis of foreign body infection is the adhesion of the micro-organisms to the biomaterial surfaces followed to the colonisation. In order to achieve a deeper understanding of the molecular-level interactions between catheters and biological system, the aim of this work is to study the physicochemical properties of the polymeric surface and their influence on the microbial adhesion and colonisation. Some common polymers produced by standard processing methods have been analysed by dynamic contact angle (DCA), X-ray Photoelectron Spectroscopy (XPS), Time of Flight Sims (TOF Sims) and Scanning electron microscope (SEM). Moreover biological tests were performed to determine the degree of gram+ and gram- bacterial adhesion on these surfaces. The results show relevant deviation of the contact angles from the expected values. These results are only partially explained by the XPS and the TOF Sims analysis. XPS and TOF Sims spectra revealed normally contaminated polymer surfaces and deviations from the nominal composition also after accurate cleaning, performed using different methods. In particular oxidation of the polymer surface occurring probably during the moulding process and other factors are able to introduce chemical functions which lead to a surface chemistry significantly different from the expected one. High value of contact angle and unexpected values of bacteria adhesion can be explained taking into account the presence of basic functions and the roughness of the surface. These elements decrease the differences expected on the basis of the acid, basic or dispersive characters of the examined polymers.

BI-WeP18 Functionalised Plasma Polymers for Control of Cell Attachment, ***J.M. Kelly,*** *R. Daw, R.D. Short,* University of Sheffield, UK

Surface chemistry is known to be an important factor in mediating cell attachment and subsequent activity on materials whether in the context of an in vitro culture system, implanted biomaterial or tissue engineered construct. In our studies on alcohol and carbonyl containing surfaces, cell attachment increased almost linearly with functional group concentration (up to 20-30%) whilst on carboxyl containing surfaces a low threshold concentration (approximately 5%) promoted excellent levels of cell attachment above which further increase of carboxyl concentration does not appear to promote greater cell attachment. Using a binary mixture of functional monomer (acrylic acid, methyl vinyl ketone or allyl alcohol) with a diluent hydrocarbon monomer (octa-1,7-diene), thin layers of polymer have been produced by radio frequency (RF) plasma deposition. Polymerisation took place at a pressure of 4x10@super -2@ mbar with the plasma supported by a 13.56 MHz, 2 W continuous wave power supply. X-Ray Photoelectron spectroscopy has shown the surfaces to contain hydrocarbon as well as hydroxyl, carbonyl and carboxyl functionality and that increasing the amount of functional monomer used in the plasma led to an increase of the corresponding functionality in the deposit. A rat osteosarcoma cell line (ROS 17/2.8) and human bone marrow cells were used for cell attachment studies. Cells were seeded at 3x10@super 4@ cells/cm@super 2@ and incubated for 3 h and 24 h in serum containing media. Cell attachment was quantified by direct counting after staining with methylene blue. Cell attachment studies are being carried out in parallel on self assembled monolayers containing similar ranges of functionality to allow comparison of plasma polymerised deposits with model surfaces.

BI-WeP19 Study of Protein Adsorption on Hydrophilic and Hydrophobic Polysiloxane Surfaces Modified by O@sub 2@ Plasma Technique, ***C. Satriano,*** University of Catania, Italy, Sweden*; F. Höök,* Chalmers University of Technology and Göteborg University, Sweden*; G. Marletta,* University of Catania, Italy*; B. Kasemo,* Chalmers University of Technology, Sweden

Thin films of a poly(hydroxymethyl)siloxane have been chemically modified by using the O@sub 2@ plasma technique at increasing treatment times ranging from 15 seconds to 10 minutes, with the applied power of 100 Watts and a residual gas pressure of 0.25 torr. The plasma-induced surface chemical modifications were investigated in situ by means of X-Ray Photoelectron Spectroscopy (XPS) for both the in situ samples and the samples aged in air and/or in water. By means of Static Contact Angle measurements the wettability properties of the unmodified and the O@sub 2@ plasma-exposed surfaces were investigated. The XPS results show that the compositional modification involves a dramatic decrease in the carbon content and the formation of [SiO@sub 4@] clusters, indicating a successive transition with treatment time from the original [SiO@sub 3@C] structure to [SiO@sub x@C@sub y@] phases. The contact angle measurements indicate that the plasma treatment changes the initial hydrophobic surface (@theta@ ~ 90°) of the polymer into a completely wettable surface, with @theta@ = 0-10° contact angle. After aging in water the surface is still completely wettable, while aging in ambient atmosphere produces a mild recovery in the contact angle values. These surfaces are currently subject to measurements of protein adsorption and of vesicle to supported membrane transformations, using QCM-D and other techniques. The results of these measurements and how they correlate with the XPS and wetting angle measurements will be presented.

BI-WeP20 Colloidal Lithographic Methods for Cell Culture Experiments, ***A.S. Andersson,*** *D.S. Sutherland, P. Hanarp, B. Kasemo,* Chalmers University of Technology, Sweden

The interaction of cells with surfaces can be modulated by the surface topography and chemistry on the micrometer and submicron length scale. Relatively recent evidence has shown that topography and chemistry on the nanometre scale can influence the funct ional behaviour of both protein molecules and selected cells. It is likely that the structural properties of a surface from the micron down to the nanometre and molecular scale are able to influence cellular behaviour, either directly or via an adsorbed hydrated protein layer. In order to systematically study the influence of surface properties on cellular behaviour methods to fabricate surfaces with defined and varied chemical and topographic architectures on a range of different length scales are requi red. A prime requirement of fabrication for cell culture experiments is that (by the standards of nanofabrication) extremely large areas of surface can be quickly fabricated. Colloidal lithographic methods have been developed to systematically fabricate nanometer features with defined size, shape and distribution over large areas in a single fabrication process. These methods utilise individual colloidal particles as a mask material for lithographic processing and have been used to create surfaces with a single type of topographic or chemical feature of defined size (available size in the range 10-200nm in all spatial dimensions). In combination with traditional photolithographic methods micrometer sized strips have been patterned with the nanometre-sized features to create surfaces with hierarchical chemical and topographic structures. These surfaces have been used in range of exploratory cell culture experiments.

BI-WeP21 Endothelial Cell Organization on Micropatterned Protein Surfaces, ***R. Daw,*** *T.N. Wight,* University of Washington*; R.B. Vernon,* Hope Heart Institute*; P.S. Stayton,* University of Washington

We have employed microcontact printing to investigate how spatial control of adhesive domains can direct the development of endothelial cell tubes for applications in tissue engineering and array-based sensors. Previous studies by Dike and co-workers showed that controlling adhesive geometries can dramatically affect endothelial cell fate and tube formation.@footnote 1@ Initial studies were directed toward comparing bovine aortic endothelial cell adhesion and activation on 5, 15 and 30 µm lanes of fibronectin (FN) versus laminin (LM) in the presence of 1 ng/ml of VEGF. Cells on LM tracks were able to migrate into intervening spaces of 20 µmm after 24 h. When the spaces between the lanes were increased from 20 to 80 µm cells remained adherent to the LM tracks except for those on the 5 µm tracks. Here, cells could spread between adjacent lanes. Endothelial cells were adherent to FN lanes throughout the range of pattern dimensions. A higher concentration of VEGF (10 ng/ml) stimulated migration off the patterned FN lines. FN lanes of 5, 15 or 30 µm were selected for subsequent studies directed toward defining the dimensionality of endothelial cell organisation into tubes. TEM showed that cells on tracks of 5 µm exhibited a significant arc of curvature and single endothelial cells encircled an organised fibrillar material to form tubes. Single tubes were also observed on 15 µm tracks but at this lane width, 2-3 cells organised together and circled a larger central fibrillar tube. These studies suggest that the composition of matrix proteins may play an important role in controlling endothelial cell development in confined geometries and that the organisation of endothelial cells into tube structures can be readily manipulated by controlling adhesive pattern dimensions. @FootnoteText@ @footnote 1@ Dike, L.E., Chen, C.S., Mrkisch, J.T., Whitesides, G.M., Ingber, D.E.; In Vitro Cell Devel. Biol. - Anim.; 35: 441-448; September 1999.

Biomaterial Interfaces
Room 202 - Session BI-WeA

Non-fouling Surfaces
Moderator: M. Grunze, University of Heidelberg

2:00pm **BI-WeA1 Fundamental Studies of Self-Assembling Monolayers as Model Systems for Biological Interfaces**, ***G. Hähner,*** ETH Zurich Switzerland, Switzerland **INVITED**

The first level of concern of interactions between proteins and synthetic surfaces deals with non-specific adsorption: that is, adsorption reflecting hydrophobic or electrostatic interaction. Chemically modified Scanning Force Microscope (SFM) probes allow it to study such interactions with surfaces separately and hence to mimic the different contributing forces to protein-surface interaction independently. Poly(ethylene glycol) (PEG) has been recognized for a long time for its outstanding protein resistant properties. The force between oligo(ethylene glycol) terminated (self-assembled) monolayers and proteins, however, depends on the conformation of the adsorbed molecules and parameters of the environment. We have studied the interaction between differently functionalized (charged and hydrophobic) SFM probes and oligo(ethylene glycol) terminated surfaces depending on the length of the ethylene glycol part, the cation in the electrolyte solution and the ion concentration of the aqueous environment.

2:40pm **BI-WeA3 Protein Adsorption and Cellular Responses to Polysaccharide Coatings**, ***S.L. McArthur,*** CSIRO Molecular Science and CRCERT, Australia*; P.G. Hartley,* CSIRO Molecular Science, Australia*; K.M. McLean, G. Johnson, M.L. Jenkins, H.J. Griesser,* CSIRO Molecular Science and CRCERT, Australia

There is increasing evidence that the cellular response to a biomedical implant is determined by the presence of specific proteins at the interface. There are a number of protein/surface interactions that influence such adsorption events. In this study we assess the roles of steric and electrostatic interactions on the protein adsorption characteristics of a range of cell supporting and cell-resistant polysaccharide surfaces. Polysaccharides were grafted onto highly anionic, flat, radio frequency glow discharge (rfgd) coatings with or without secondary graft supporting interlayers. Polysaccharides of differing functionality and charge density (aminodextran, carboxymethyldextran and oxidised dextran) were used. The properties of the resulting surfaces were assessed using XPS, streaming potential and AFM imaging and colloid probe force measurements. The impact of surface properties on protein adsorption was also monitored using XPS, ELISA and Surface-MALDI-MS techniques. The results of these studies indicate that protein adsorption occurs regardless of steric interactions provided an electrostatic attraction exists between the protein and the surface. To illustrate this point, surfaces were engineered which were expected to display a low affinity for cell adhesive proteins. In vitro cell studies on such surfaces showed minimal cell attachment and growth which was in marked contrast to the same polysaccharide surfaces with the proteins covalently attached. In this instance, cell attachment and growth was observed. These studies demonstrate the role of specific protein adsorption in the mediation of cellular responses on polysaccharide surfaces.

3:00pm **BI-WeA4 Film Formation of Self-assembled Monolayers of Thiol-modified Polyethylene Glycol on Gold**, ***S. Tokumitsu,*** *S. Herrwerth, W. Eck, M. Himmelhaus, M. Grunze,* Universität Heidelberg, Germany

The adsorption kinetics of self-assembled monolayer (SAM) of thiol-modified polyethylene glycol (PEG: 2000 dalton) on polycrystalline gold surface in dilute solution has been studied by using ellipsometry, infrared reflection absorption spectroscopy (IRRAS), X-ray photoemission spectroscopy (XPS) and second harmonic generation (SHG). Thickness and structure of the finally obtained layer exhibit strong dependence on the solvent. In-situ SHG monitoring of the headgroup adsorption of PEG from 50 μM DMF solution shows rapid coverage increase up to 44% within the first 10 min of immersion, followed by a slowly proceeding adsorption step. Final coverage is reached after a total immersion time of about 2 hours. On the other hand, ellipsometry reveals that film thickness drastically increases after 1-2 hours of immersion. These results indicate a restructuring of the PEG moiety from an amorphous to a crystalline-like phase at that time. The results for film thickness and structure obtained by in-situ and ex-situ experiments will be discussed in detail and finally a phenomenological model of the adsorption process will be presented.

3:20pm **BI-WeA5 Synthesis and Characterization of Functionalized Polymerizable Diacetylene Containing Thiol Monolayers**, ***N.R. Holcomb,*** Agilent Technologies*; D.G. Castner,* University of Washington*; D.W. Grainger,* Colorado State University

Self-assembled structures have been used to tailor the surface character of materials: enhance adhesion, lubrication, catalysis, and molecular/cell immobilization and recognition in biotechnology.@footnote 1,2@ Utilization of polymeric materials in the self-assembling monolayer can increase the thermal and mechanical strength of the films formed from monomeric materials.@footnote 3@ The use of polymeric blocks within self-assembled films has shown that there is added stability toward solvent attack.@footnote 4,5@ We report the synthesis and characterization of new surface functional diacetylene thiols of the formula: R-(CH2)n-C?C-C?C-(CH2)m-SH where n=m=9, and R=fluoro alkyl, or oligoethylene glycol. These materials were synthesized via multistep schemes featuring an asymmetric Cadiot-Chodkiewicz coupling to create the functional diacetylenes from pairs of alkynes. Self-assembled monolayers of these organic molecules are characterized with polarized FTIR, ellipsometry, contact angle, XPS and TOF-SIMS. Films were formed from diacetylene thiols containing contrasting surface functional groups to provide a triethylene glycol non-biofouling surface@footnote 6@ and a low surface energy fluorinated surface.@footnote 3@ @FootnoteText@ @footnote 1@ K. M. McClary, D. W. Grainger, Biomaterials, 20, 1999, 2435-2446 @footnote 2@ K. M. McClary, D. W. Grainger J. Biomed. Mater. Res., in press (2000). @footnote 3@ Ebert, R.; Laschewsky, A.; Ringsdorf, H.; J. Am. Chem. Soc. 1985, 107, 4134 @footnote 4@ Sun, F.; Grainger, D.W.; J. Polym Sci. Polym. Chem. Ed. 1993, 31, 172 @footnote 5@ Sun, F.; Castner, D.G.;Grainger, D.W.; Langmuir. 1993, 9, 3200 @footnote 6@ Pertsin, A. J.; Grunze, M.; Garbuzova, I. A. J. Phys. Chem. B 1998, 102, 4918-4926.

3:40pm **BI-WeA6 Modification of Metal Oxide Surfaces for Biosensor and Biomaterial Applications Based on Assembled, Functionalized Poly(L-lysine)-*g*-poly(ethylene glycol)**, ***M. Textor,*** *J. Vörös, R. Hofer, D. Elbert,* ETH Zurich, Switzerland

Poly(L-lysine) grafted with poly(ethylene oxide) (PLL-g-PEG) is a polycationic block copolymer that spontaneously assembles as a monolayer at negatively charged metal oxide surfaces such as those formed by titanium oxide, tantalum oxide or niobium oxide. The interaction with the negatively charged surface is shown to be electrostatic through the terminal amine groups of the poly(L-lysine) side chains charged positively at pH below 9. The surfaces have been characterized ex situ using X-ray photoelectron spectrocopy, time-of-flight secondary ion mass spectrometry and reflection-absorption infrared spectroscopy. The planar optical waveguide (grating coupler) technique was used in situ both to monitor in real time the assembly process at the metal oxide waveguide surface, as well as to determine the degree of non-specific adsorption when exposed to serum. The degree of protein resistance was found to depend on the PLL-g-PEG coverage, on the grafting ratio between lysine monomer units and PEG side chains, and on the molecular weight of the PEG used. Using optimized polymer architectures, very low values of serum adsorption could be achieved, typically below the detection limit of our optical waveguide instrument (1 ng/cm2). The surfaces remain protein-resistant in flowing buffer solution at least up to 7 days. Functionalized PLL-g-PEG molecules were synthesized with functional groups such as biotin at the terminal position of the PEG side chains. The functionality of these polymer layers on optical waveguide chips was investigated using a model assay with streptavidin binding, followed by the adsorption of biotinylated recognition units and targeting of proteins such as IgG. This new polymeric interface is shown to have an excellent potential for future applications both in the area of bioaffinity sensor to control specific and non-specific adsorption and for implants such as stents.

4:00pm **BI-WeA7 Design and Characterization of Specific Biorecognition Interfaces using Derivatized Poly(L-lysine)-grafted-poly(ethylene glycol) Monolayers**, ***L.A. Ruiz-Taylor,*** *T.L. Martin, M. Heidecker, P. Indermuhle, P. Wagner,* Zyomyx, Inc.

Control of interfacial events such as specific recognition versus non-specific protein adsorption is a major issue in biotechnological applications. In diagnostic assays or biomaterial devices, non-specific binding events can often be the limiting factor towards higher detection sensitivity or implant integration, respectively. In this study, we report the design of interfacial polymers that have the ability to spontaneously adsorb to negatively charged surfaces under physiological pH and efficiently repel non-specific protein adsorption while providing PEG tethered functional/active sites for specific biomolecule recognition. As a model system, we synthesized

biotin-derivatized poly(L-lysine)-grafted-poly(ethylene glycol) copolymers, PLL-g-(PEG)(1-x)(PEG-biotin)x, where x varies from 0.1 to 1. XPS was used to characterize the properties and the organization of the monolayers formed on titanium dioxide. Molecular recognition properties were investigated using radiolabelled streptavidin alone and within complex protein mixtures. We showed that the system allows the specific recognition of streptavidin, that the extent of the recognition is not influenced by the presence of other proteins and that streptavidin-horseradish peroxidase displays enzymatic activity on the modified surfaces. Finally, we used the PLLPEG-biotin copolymer system in conjunction with microfluidic patterning techniques to provide micron-size features with specific protein recognition separated by areas preventing non-specific binding, as shown by AFM and fluorescence microscopy.

4:20pm **BI-WeA8 Analysis of Protein Absorption on PEG-covered Silica Surfaces by ATR-FTIR**, *N.A. Alcantar,* ***A. Stacy***, *J. Au,* University of California, Santa Barbara*; T.L. Kuhl,* University of California, Davis*; E.S. Aydil, J.N. Israelachvili,* University of California, Santa Barbara

The most desirable characteristic of biomaterials is their capability to reject protein adhesion because non-specific adsorption of proteins to a surface of an artificial material enhances atypical development of cells. Surfaces covered with polyethylene glycol (PEG, OH-(CH@sub 2@-CH@sub 2@-O)@sub n@-H) have been shown to enhance protein rejection, nonimmunogenecity and nonantigenicity. In order to produce a generic biocompatible surface coating, we developed and analyzed a direct method for grafting PEG onto amorphous water plasma activated silica surfaces or films. In this paper, we investigated the biocompatibility of this PEG coating by measuring its ability to resist protein adsorption with attenuated total internal reflection Fourier transform infrared (ATR-FTIR) spectroscopy. PEG-coated silica surfaces, water plasma treated silica surfaces, and bare silica films were all exposed to several concentration solutions of fibrinogen and human serum albumin (HSA) at 37°C and pH=7.4 (imitating physiological conditions). We measured the protein adhesion to each of these three surfaces. We found that the surface covered with PEG had very little protein adsorption. Conversely, the bare silica surface has relatively high amounts of adsorbed protein. The surface treated with water plasma (but no polymer) adsorbed some proteins falling in between the bare silica and PEG-coated surfaces. PEG-covered silica coatings can be applied to protect diverse materials having different chemistries and shapes.

4:40pm **BI-WeA9 Combining Polymer Chemistry and Photolithography to Manipulate Gene Expression and Protein Synthesis**, ***K.E. Healy***, University of California, Berkeley, U.S.A*; J.H. Collier, C.H. Thomas,* Northwestern University*; C. Sfeir,* OHSU*; S.L. Golledge, D.G. Castner,* University of Washington

Materials that actively regulate the response of mammalian cells are designed to act via a combination of biomolecular recognition processes and device microarchitecture. We have developed methods that incorporate photolithography, organosilane chemistry, photoinitiated polymerization, and peptide chemistry to create surfaces that control the spatial distribution, projected area, and nuclear shape of mammalian cells. Interfacial interpenetrating polymer networks (IPNs) were synthesized by sequential photoinitiated free-radical polymerization of a thin layer of polyacrylamide followed by a secondary photoinitiation step using poly(ethylene glycol)-based monomers to create the network. Characterization of the IPNs by contact angle goniometry, spectroscopic ellipsometry, XPS, and static SIMS has confirmed the formation of an interfacial IPN ~ 20nm thick. These IPNs prevent protein adsorption and cell adhesion and therefore represent an excellent surface to control the spatial distribution of either biological macromolecules, cells, or viruses. In one application, materials with patterned surface chemistry could serve as templates for the organization of tissue structure surrounding medical devices, which would theoretically influence their biocompatibility. To address this hypothesis, the nuclear shape of mammalian cells was controlled on microfabricated substrata with reigospecific chemistry. Protein synthesis and expression at the mRNA level and were altered by changing the shape of the cell nucleus. Our data supports the concept of “architectural” transcription factors that promote gene expression based on optimal stress within the nuclear matrix transduced by the cytoskeleton.

5:00pm **BI-WeA10 Investigation of Protein Interactions with Poly (Ethylene Glycol) Modified Liposomes**, ***J.L. Brash***, *M.E. Price,* McMaster University, Canada

Liposomes have considerable potential as drug delivery vehicles. However unmodified liposomes are rapidly removed from the circulation by the reticuloendothelial system. This is believed to be initiated by adsorption of plasma proteins. Modification of liposomes with poly(ethylene glycol) (PEG) has been shown to increase their lifetime in vivo. Although there is some information on protein interactions with conventional liposomes, there is little if any on PEG-modified liposomes. In this study liposome interactions with fibrinogen in buffer and with plasma have been investigated. Sucrose-loaded large unilamellar liposomes were prepared. Dry phospholipid films (unmodified or modified with phosphatidylethanolamine-conjugated PEG (PE-PEG)) were hydrated with sucrose buffer followed by extrusion through two stacked 100 nm polycarbonate membranes. Liposome size distribution was estimated by dynamic light scattering (DLS) with diameters in the range of 135 ± 8 nm. Liposomes were incubated for 3 h in Tris-buffered 125I-fibrinogen solutions. The mixtures were centrifuged and the radioactivity of liposome pellet determined. Liposomes were also incubated in plasma and bound proteins identified by SDS solubilization followed by gel electrophoresis and immunoblotting using antibodies to some 20 plasma proteins. Fibrinogen adsorption was found to increase as solution concentration increased, with no apparent plateau. Adsorbed amounts decreased with incorporation of PEG and with increasing MW of PEG in the range 500-5000. The gels and immunoblots showed that the unmodified and PEG-modified liposomes adsorbed most of the proteins probed for. The protein patterns (relative amounts of each, degradation, activation) were similar.

Biomaterial Interfaces
Room 202 - Session BI+NS-ThM

Nanoscale Biology
Moderator: J.J. Hickman, Clemson University

8:20am **BI+NS-ThM1 Engineering Life into Nanofabricated Systems*, C.D. Montemagno,*** Cornell University **INVITED**

Scientists and engineers have anticipated the potential benefits of integrating engineered devices to living systems at the molecular level for many years. Hybrid systems can potentially possess many of the essential properties of life such as the abilities to "intelligently" self-assemble, repair, and evolve. We will present the results of our efforts to incorporate biological energy transduction processes and cell signaling pathways into engineered nanofabricated devices. In particular, we will illustrate our strategy for fueling, controlling and integrating a F1-ATPase biomolecular motor with a NEMS to create an engineered hybrid device. Included in the presentation will be the initial results of our efforts to develop and demonstrate an integrated F1-ATPase powered NEMS device that is fueled by light-driven ATP production. ATP is synthesized from light using artificial liposomes comprised of reconstituted FoF1-ATP synthase and bacteriorhodopsin. Subsequently, the ATP provides energy to power a recombinant, thermostable F1-ATPase biomolecular motor that is coupled to a NEMS device. We will also present our technique for integrating nanomechanical structures to biomolecular motors with a precision d 40 nm. This work capitalizes on a core feature of living systems: the capability of transforming diverse sources of energy into a generic energy currency that can be universally used. The integration of a synthetic photosynthetic system with NEMS establishes a new mechanism for fueling the next generation of nanoelectromechanical devices. Light is used to produce ATP from ADP and P, the ATP is used by the F1-ATPase biomolecular motor to produce work with ADP and P as waste products. Ultimately, we anticipate that this chemically closed system will be used to pump fluids, open and close microvalves, provide locomotion, generate electricity, and make way for "Smart Dust" applications such as long-lived microscopic intelligence and environmental sensors.

9:00am **BI+NS-ThM3 Powering Molecular Shuttles through an Artificial Photosynthetic System*, V. Vogel, H. Hess,*** University of Washington*; K. Jardine,* Arizona State University*; J. Clemmens,* University of Washington*; T.A. Moore, A.L. Moore, A. Primak,* Arizona State University*; J. Howard,* University of Washington*; D. Gust,* Arizona State University

The ultimate goal for bioengineers is to be able to engineer systems on a nanoscale as perfect as nature does in cells. Great progress has been made in recent years in biochemistry and biophysics, supplying us with information about the construction principles as well as the details of many cellular subsystems. This information is matched by recent advances in nanotechnology, allowing control of the arrangement of biomolecules on a sub-micron scale. From an engineering point of view the construction of artificial systems, performing different tasks related to the cellular environment, becomes possible. Examples of this approach are the construction of artificial photosystems,@footnote 1,2@ consisting of vesicles doped with antenna molecules, proton pumps and the enzyme ATPase, and the construction of "molecular shuttles",@footnote 3,4@ microtubules moved by motor proteins on a patterned surface. The arising challenge is to combine these subsystems into a larger, more complex system with extended functionality. Here we present a proof-of-principle experiment demonstrating the integration of a transport systems (the "molecular shuttles") with a system providing chemical energy from light (the above mentioned artificial photosystem). In the integrated system we can therefore nonintrusively control the motion of the microtubules through light. The experimental setup consists of a flow cell mounted on an epi-fluorescence optical microscope and illuminated by a laser diode. The surface of the flow cell was patterned with parallel grooves spaced between 30 nm and 1 um apart by shear-deposition of a teflon film@footnote 5@. The motor protein kinesin@footnote 6@ adsorbed preferentially along the grooves providing "tracks" for the motion of the microtubules. The microtubules were fluorescently labeled and bound to the motor proteins in the absence of ATP. The ATP-generating vesicles floated freely in the buffer solution. Illumination of the sample with light absorbed by the vesicles as followed by motion of the microtubules. The motion was mainly directed along the direction of shear of the underlying teflon film. This experiment thus demonstrated that in an integrated system, multiple self-assembled entities cooperate functionally all the way from light harvesting through charge separation across a lipid membrane and ATP-synthesis driven by a proton gradient to ATP-fueled conformational changes of kinesin leading to directed motion of microtubules on uniaxially aligned kinesin tracks. @FootnoteText@ @footnote 1@ Gust, D., T.A. Moore, and A.L. Moore, Mimicking bacterial photosynthesis. Pure & Appl. Chem., 1998. 70(11): p. 2189-2200. @footnote 2@ Steinberg-Yfrach, G., et al., Light-driven production of ATP catalysed by F0F1-ATP synthase in an artificial photosynthetic membrane. Nature, 1998. 392(6675): p. 479-82. @footnote 3@ Dennis, J.R., J. Howard, and V. Vogel, Molecular shuttles: directing the motion of microtubules on nanoscale kinesin tracks. Nanotechnology, 1999: p. 232-236. @footnote 4@ Service, R.F., Borrowing from biology to power the petite: nanotechnology researchers are harvesting molecular motors from cells in hopes of using them to drive nano-scale devices. Science, 1999. 283: p. 27-28. @footnote 5@ Wittmann, J.C. and P. Smith, Highly oriented thin films of poly(tetrafluoroethylene) as a substrate for oriented growth of materials. Nature, 1991. 352: p. 414-417. @footnote 6@ Howard, J., A.J. Hudspeth, and R.D. Vale, Movement of microtubules by single kinesin molecules. Nature, 1989. 342: p. 154-158.

9:20am **BI+NS-ThM4 Unbinding Process of Adsorbed Proteins under External Stress Studied by AFM Force Spectroscopy*, C. Gergely, J. Voegel,*** INSERM, France*; P. Schaaf,* Institut Charles Sadron (CNRS) Strasbourg, France*; B. Senger,* INSERM, France*; J.K.H. Horber,* EMBL Heidelberg, Germany*; J. Hemmerle,* INSERM, France

We report the study of the unbinding process under a force load f of adsorbed proteins (fibrinogen) on a solid surface (hydrophilic silica) by means of AFM force spectroscopy. By varying the loading rate r, defined by f=r.t, t being the time, we find that, as for specific interactions, the mean rupture force increases with r. This unbinding process is analysed in the framework of the widely used Bell model. Thus typical dissociation rate at zero force entering in the model lies between 0.02 and 0.6 1/s. Each measured rupture is characterized by a force f0 which appears to be quantized in integer multiples of 180-200 pN.

9:40am **BI+NS-ThM5 Single-Molecule Protein-Ligand Bond-Rupture Forces Measured Using the Poisson Atomic Force Method*, Y.-S. Lo, Y.J. Zhu, J.D. McBride, T.P. Beebe, Jr.,*** University of Utah **INVITED**

It is known that bond strength is a dynamic property that is dependent upon the force loading rate applied during the rupturing of a bond. For biotin-avidin and biotin-streptavidin systems, dynamic force spectra, which are plots of bond strength vs. ln(loading rate), have been acquired in a recent biomembrane force probe (BFP) study [Merkel et al., Nature 397 (1999) 50] at force loading rates in the range of 0.05 to 60,000 pN/s. In the present study, the dynamic force spectrum of the biotin-streptavidin bond strength in solution was extended from loading rates of ~10@super 5@ to ~10@super 9@ pN/s with the atomic force microscope (AFM). The Poisson AFM statistical analysis method was applied to extract the magnitude of individual bond-rupture forces and non-specific long-range interactions from the AFM force-distance curve measurements. In addition, surface characterization methods for the analysis of protein-coated surfaces and AFM tips, both imaging and spectroscopic x-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) will be discussed. The AFM bond strengths were found to scale linearly with the logarithm of the loading rate in two regimes with two different slopes, consistent with the view that multiple energy barriers are present along the unbinding coordinate of the biotin-streptavidin complex. In contrast, the non-specific interactions, which can be separately measured and characterized apart from the specific bond-rupture forces in this method, did not exhibit a measurable dependence on loading rate. The dynamic force spectra acquired here with the AFM combined well with BFP measurements by others, and demonstrated that unbinding forces measured by different techniques are in agreement and can be used together to obtain a dynamic force spectrum covering 11 orders of magnitude in loading rate.

10:40am **BI+NS-ThM8 Measuring the Mechanical Properties of Soft Samples by Atomic Force Microscopy*, M. Radmacher,*** Universit@um a@t G@um o@ttingen, Germany **INVITED**

The Atomic Force Microscope combines in a very unique way a very high sensitivity in detecting and applying forces (of up to a few 10's of piconewton), a high accuracy in positioning a sharp tip relativ to the sample in all three dimensions (of up to a few Angstrom), and the possibility to be operated under physiological conditions. This combination allows experiments not possible before, particularly in the field of biophysics and soft materials. One example is the mapping of mechanical properties with high spatial resolution of polymeric films and living cells. In living cells it is

possible to probe the mechanics during dynamic processes like cell migration and cell division.

11:20am **BI+NS-ThM10 Protein Adsorption and Monocyte Activation on Ge Nanopyramids**, ***B. M@um u@ller,*** ETH Z@um u@rich, Switzerland; *M. Riedel,* ProBioGen, Germany; *R. Hofer, E. Wintermantel,* ETH Z@um u@rich, Switzerland

The performance of an implant material depends crucially on its surface architecture or morphology. The significance of topographic features with micrometer size on cell shape and function has been clearly demonstrated. The power of features on the nanometer scale is still under discussion. In order to get an insight into the responds of monocytes onto a well-defined substrate nanostructure, we have grown germanium nanopyramids on Si(100) in a natural way by epitaxial growth, i.e. without any lithographic technique. The density of the pyramids (hut and dome cluster) is adjusted varying the substrate temperature during deposition. The morphology of the oxidized pyramids is quantified by ex situ atomic force microscopy. To characterize the nanostructure roughness further, contact angles of water under dynamic conditions are measured in comparison with the bare Si wafer and flat Ge films on Si. The receding angles show a significant increase with pyramid density. The amount of the selected proteins g-globulin and albumin adsorbed on the nanostructures is determined spectroscopically with labeled proteins. It raises with pyramid density. The impact of nanostructuring on the biological activity of adsorbed g-globulin is addressed by immunosorption with an anti-g-globulin antibody. These data reveal that the amount of active g-globulin does not scale with the adsorbed one. Nanoscale roughness even decreases the activity. The in vitro assays with monocytes that allow studying specific aspects of inflammatory reactions of the body - an important aspect of the biocompatibility, are based on the monocyte-like cell line U937. After 5 days in vitro, the cell performance is characterized microscopically and by the secreted cytokines IL-1b, IL-1ra and TNF-a. For the CVD grown samples, a roughness increase leads to reduced cytokine expression. Consequently, implants with nanoscale roughness gives rise to less inflammatory reactions.

MEMS

Room 309 - Session MM+BI-ThM

Bio-MEMS and Microfluidics

Moderator: P.G. Datskos, Oak Ridge National Laboratory

8:20am **MM+BI-ThM1 Microfabrication Technologies for Biomedical Applications**, ***M. Ferrari,*** The Ohio State University **INVITED**

PLEASE SEND US AN ABSTRACT. Thank you.

9:00am **MM+BI-ThM3 Suppression of Blood Serum Adhesion on Quartz Inner Wall of Microcapillary Coated by Bio-compatible MPC Polymer**, *H. Ogawa, A. Oki,* ***Y. Takamura,*** *S. Adachi,* The University of Tokyo, Japan; *T. Ichiki,* Toyo University, Japan; *K. Ishihara, Y. Horiike,* The University of Tokyo, Japan

We are developing the healthcare device which enables us to detect various health markers from trace amount of the blood. To suppress adhesion of proteins in a blood injected into a quartz made microcapillary, the 2-methacryloyloxyethylphosphorylcholine (MPC) polymer which is now utilized to artificial blood tube, contact lens, in-vivo sensors, etc. has been coated on the inner wall, thus leading to bio-compatibility of the quartz surface. A microcapillary with a 30 x 30 µm cross-section and 10 mm in length was fabricated in a 2cm x 2cm quartz chip by dry-etching and subsequent press-bonding of a cover quartz plate in 1% HF solution. A pH=7.4 phosphate buffered solution (PBS) was filled in the capillary and the serum was injected from one end by electroosmosis pumping. The concentrations of proteins, which were monitored by 220nm UV absorption at the point of 4mm apart from the injection, rose up rapidly when the serum arrived at this point and then were kept at a constant value in the 3 wt% MPC coated capillary, while those in uncoated capillary were decreased gradually by adhesion of proteins on the inner wall after showed maximum. To investigate the interaction of proteins with the MPC polymer coated surface, FTIR-ATR spectra for MPC coated and uncoated wall surfaces exposed by a blood serum were measured. The adsorption peaks by NH@sub x@ and C=O of proteins in the uncoated surface increased with exposure time, while the proteins did not absorb on the coated surface. These results clearly demonstrate the excellent bio-compatiblity of the MPC polymer for a blood handling capillary chip.

9:20am **MM+BI-ThM4 Biologically-Compatible Polymeric MEMS Devices Fabricated using Holographic Two-Photon Induced Photopolymerization**, ***L.L. Brott,*** Technical Management Concepts, Inc.; *S.M. Kirkpatrick,* Science Applications International Corporation; *M.O. Stone,* Wright-Patterson Air Force Base

Research in the bio-MEMS field has been driven by the desire to reduce the time, complexity and equipment needed to carry out clinical diagnostic procedures. Current bio-MEMS strategies rely extensively upon externally added reagents and conventional photolithography in the fabrication of these systems. In an effort to simplify these devices even further, research has begun incorporating the reagents, i.e. enzymes, directly into the walls of the microfluidic channels. Consequently, a new polymeric material based on poly(ethylene glycol) which maintains the biological activity of the reagents and is not sensitive to aqueous environments, yet whose monomeric form accepts aqueous solutions, was developed. The microfluidic channels were patterned by photocuring the monomer using a two-photon initiated photopolymerization process at 800 nm. A rose bengal/triethanol amine initiator system was used. By using a laser as the light source, holograms were patterned onto the device resulting in well defined and complex patterns.

9:40am **MM+BI-ThM5 Patch Clamping with Microfabricated Planar Electrodes**, ***K.G. Klemic,*** *J.F. Klemic, M.A. Reed, F.J. Sigworth,* Yale University

The patch clamp technique is the most sensitive way to record the small ionic currents carried by ion channels and transporters in cell membranes. To make a typical recording, the ~1 micron tip of a glass or fused-quartz micropipette, filled with saline solution, is sealed over a patch of cell membrane, electrically isolating it with a very large electrical "seal resistance" of 10-100 G@ohm@. Here we describe the first use of new materials and a new configuration for patch-clamp electrodes. We have microfabricated planar electrodes that mimic the shape of the tip of the micropipette by aniostropic etching of single crystal quartz or by micromolding the silicone elastomer, poly(dimethylsiloxane) (PDMS). The planar geometry has several advantages over the standard glass micropipette currently used for patch clamp recording. First, it permits direct integration of the first stage of amplification electronics into the electrode. Second one electrode can be easily scaled to an array of electrodes for simultaneous patch-clamp recordings from many cells, greatly expanding the discovery of new ion channel genes and new pharmacological agents directed to ion channel targets. Third, microfluidic channels can be integrated to permit fast solution exchange on both sides of the membrane, something that is not presently possible. Lastly, the electrode is designed to have a small solution volume that reduces the capacitance and thereby reduces by an order of magnitude the contribution of the electrode to the background noise. The design and fabrication of these novel patch electrodes as well as membrane current recordings using these devices will be presented.

10:00am **MM+BI-ThM6 High Sensitivity Resonant Biosensor**, ***B. Ilic,*** *D. Czaplewski, H.G. Craighead,* Cornell University; *P. Neuzil,* Institute of Microelectronics, Singapore; *C. Campagnolo, C. Batt,* Cornell University

There is a growing demand to produce highly selective biological sensors for the detection of small quantities of biological microorganisms using micromachining. In this work, the detection of bacteria using a resonant frequency based mass detection biological sensor has been accomplished. The biological sensor under development consists of an array of resonating cantilever beams fabricated, using bulk silicon micromachining techniques, from both low pressure chemical deposition (LPCVD) and plasma enhanced chemical vapor deposition (PECVD) low stress silicon nitride. For this experiment an array of cantilevers with dimensions of varying length from 15µm to 500µm, varying width of 2µm to 20µm, thickness of 320nm for the LPCVD, and t=600nm for the PECVD nitride, were used. Escherichia coli O157:H7 antibodies were immobilized on the surface of the resonators. Devices were subsequently exposed to varying concentrations of E. Coli cells in solution and any loosely bound cells were removed. In order to determine the mass bound to the cantilever, a frequency spectra was taken before and after the binding of the cells to the cantilever. Signal transduction of the micromechanical oscillators has been accomplished by measuring the out of plane vibrational resonant mode with an optical deflection system. The measured vibrational mode was entirely due to thermal noise and ambient vibrations in air. The measured resonant frequency shift as a function of the binding of additional cells was observed and correlated to the mass of the specifically bound E. Coli O157:H7 cells. Our results indicate good agreement with the predicted theory of linear elasticity. Under ambient conditions where considerable damping occurs,

we were able to detect single E. Coli cells. Methods, utilizing vacuum encapsulation and tailoring of the cantilever dimensions, for single molecule detection will be discussed.

10:20am **MM+BI-ThM7 Nanofluidic Entropic Trap Array device for DNA Separation**, ***J Han***, *H.G. Craighead,* Cornell University

A nanofluidic entropic trap array device@footnote 1@ for separation and analysis of long DNA molecules was constructed and tested. The device contains many constrictions (entropic traps) with dimensions smaller than the radius of gyration for the DNA molecules. The length dependent trapping of DNA and resulting electrophoretic mobility difference enables efficient separation of DNA in the range of 5kb ~ 200kb, typically within 30 minutes, in a channel as short as 15 mm without using a gel.@footnote 2@ We fabricated devices with multiple channels with the same structural parameters, for a parallel analysis of samples. Multiple DNA samples were separately introduced into the device and collected into narrow bands for launching. The amount of DNA in the launching band could be electrically controlled. Simultaneous separation of multiple samples enables one to compare electrophoregrams for calibration or direct comparison for applications such as DNA fingerprinting. A range of device parameters were tried, and the optimization of the device will be discussed. @FootnoteText@ @footnote 1@ J. Han and H. G. Craighead, J. Vac. Sci. Tech. A, 17, 2142 (1999) @footnote 2@ J. Han and H. G. Craighead, Science, in publication (2000)

10:40am **MM+BI-ThM8 Integration of Microcapillary Electroporesis and Inductively Coupled Plasma Spectrometry for Rapid Biological/Chemical Analysis**, ***T. Ichiki***, *T. Koidezawa, R. Taura, T. Ujiie,* Toyo University, Japan*; Y. Horiike,* The University of Tokyo, Japan

Rapid and sensitive elemental specification of trace amounts of samples are important for chemical, biological, environmental and clinical applications. For the goal of integration of microcapillary electrophoresis (μCE) and inductively coupled plasma (ICP) optical emission spectrometry (OES) on a chip, we have fabricated μmCE chips with a nebulizer, and have investigated conditions for generating microscale ICPs. Microcapillary and nebulizer patterns were etched onto the quartz plate in C@sub 4@F@sub 8@/SF@sub 6@ plasmas using Cr masks. The etched quartz plate was dipped in 1% diluted HF solution and then bonded together with the other quartz plate with drilled holes. These two plates were press-bonded under the load of 1.3 MPa at room temperature for 24 hours. Samples separated via electroosmotic and electrophoresis phenomena were nebulized by controlling the carrier gas flow around the nozzle located at the capillary end to achieve the injection of pico-liter droplets in the gas. Subsequently, generation of microscale VHF-ICPs was investigated. Discharge chambers of 500μm-5 mm depth and/or width were fabricated on 20 mm@super 2@ quartz plates, which was attached under the 70-mm-@phi@ circular quartz plate set on a small vacuum chamber. A 5-mm-@phi@ antenna was set on the circular quartz plate so as to locate just above the discharge chamber. The power for Ar plasma ignition and mode jump from E- to H-discharge was examined by means of spectroscopy. In the case of discharge chamber dimension of 5 mm width and more than 2 mm depth, the power for ignition was only 5 W at pressures of 0.01-1 Torr, while no mode changes were observed even at 100 W. When reducing the discharge chamber depth to 1-1.5 mm, mode change occurred around 10 W and emission intensity drastically increased by 100 times. Thus high density VHF-ICP was found to be easily attained when the characteristic length of the discharge space is around 1 mm and the pressure is 1-10 Torr.

11:00am **MM+BI-ThM9 Silicone Elastomer Microwell Arrays for High Throughput Protein Biochemical Assays**, ***J.F. Klemic***, *H. Zhu, M.A. Reed, M. Snyder,* Yale University

The identification of the function of large numbers of gene products is an important challenge in post-genomic research. Inexpensive, disposable microwell arrays have been developed for high throughput screening of protein biochemical activity. The microwell arrays are cast in silicone elastomer sheets and placed on top of microscope slides for compatibility with commonly available sample handling and recording equipment. Arrays consist of high density (hundreds per slide), small volume (~300nl) wells which permit high throughput batch processing and simultaneous analysis of many individual samples using only small amounts of protein. Device utility has been demonstrated through the simultaneous analysis of 120 protein kinases from Saccharomyces cerevisiae assayed for phosphorylation of 17 different protein substrates. These microwell arrays, as tested, permit the simultaneous measurement of hundreds of protein samples, however, the use of micromolded silicone elastomer allows array densities to be readily increased by several orders of magnitude. With the further development of appropriate sample handling and measurement techniques, these arrays may be adapted for the simultaneous assay of several thousand to millions of samples.

11:20am **MM+BI-ThM10 Development of a Micro Capillary Pumped Loop System for Microelectronic Device Cooling**, ***H. Yun***, *H. Lee, K. Cho, I. Song,* Samsung Advanced Institute of Technology, South Korea

Increasing demand for processing data leads to faster clock speeds and large integration. As a result, the heat generation of microelectronic devices is increasing at rapid rate. Current PCs generate 20~30 Watts/cm@super 2@ of heat. If this trend continues, 100 Watts/cm@super 2@ of heat generation are expected within few years. Since conventional phonon diffusion based metal heat sinks can handle only up to 10 Watts/cm@super 2@, an alternative cooling technology is desired. In this paper, a micro capillary pumped loop (CPL), which is a microfabricated, capillary pressure driven fluid cycle is proposed as an alternative means of cooling microelectronic devices with large heat generation. The heat is absorbed at the evaporator by vaporizing the circulating fluid, and released out of the system at the condenser. The capillary forces of the microfabricated wick structure at the evaporator drives the fluid. Since the fluid particle directly carries the heat out of the system, the micro CPL is expected to be more effective than the conventional diffusion-based heat sinks. A prototype of 30 Watts/cm@super 2@ cooling capacity has been built and tested. Microchannels have been etched on a silicon wafer to form the evaporator and the condenser. The prototype operated successfully under 30 Watts/cm@super 2@ heat flux while keeping the junction temperature below 400K. The maximum heat flux was 50 Watts/cm@super 2@ before the dryout at the evaporator occurred. A nonlinear dynamic model has been developed to simulate the interaction between various components of the micro CPL. The simulation model successfully captured the overall trends of the experimental data. Further research on the underlying physics are desired for better understanding of this device.

11:40am **MM+BI-ThM11 Design, Fabrication, and Testing of Cross Flow Micro Heat Exchanger**, ***C.R. Harris***, *M. Despa, K.W. Kelly,* Louisiana State University

A cross flow micro heat exchanger was designed and fabricated to maximize heat transfer from a liquid to a gas (air) for a given frontal area and pressure drop of each fluid. To accomplish the goal of high heat transfer, micro channels with scales ranging from 200 μm to 500 μm were utilized. By constraining the flow to narrow channels, heat transfer is enhanced since the convective resistance at the solid/fluid interfaces is reduced. To minimize the pressure drop associated with micro channels, air passes through the plane of the heat exchanger via thousands of parallel short channels. Heat is transferred to the air from liquid that passes in cross flow through tens of parallel channels. Predicted design performance for plastic, ceramic, and aluminum micro heat exchangers are compared to one another and to current innovative car radiators. The micro heat exchangers can transfer greater heat per mass or volume than existing heat exchangers within the context of the design constraints specified. Important applications of this technology include automotive, home heating, and aerospace fields. The heat exchanger designed for plastic was fabricated by aligning and bonding two identical polymer (PMMA) parts that had been embossed using the LIGA process. After heat exchanger assembly, liquid was pumped through the heat exchanger with no leaks. Heat transfer and pressure drop tests were performed on the fabricated polymer heat exchanger. The experimental data is compared to the design calculations and to other heat exchangers.

Biomaterial Interfaces
Room 202 - Session BI+NS-ThA

Biosensors
Moderator: A. Chilkoti, Duke University

2:00pm **BI+NS-ThA1 Substrate and Attachment Chemistry Effects on Adsorption and Single-Base Mismatch Discrimination on Immobilized Oligonucleotide Arrays**, ***J.E. Forman***, *L. Gamble, R.S. Gascon, J.I. Henderson, A.D. Suseno, P. Wagner,* Zyomyx, Inc. **INVITED**

Hybridization efficiency and ability to discriminate between perfect match and single-base mismatch target sequences are fundamental to performance of arrays of covalently immobilized nucleic acids (probes). Two factors that significantly contribute to array performance are density and orientation of immobilized probes. Probe density not only controls how much bound target is adsorbed, but also affects the kinetics and thermodynamics of duplex formation. Perturbations to duplex formation occur when the spacing of probes is close enough to force inter-probe association and crowding, phenomena that interfere with target sequence adsorption. However, these perturbations can induce beneficial effects in array performance. For example, crowded nucleic acid surfaces demonstrate lower apparent melting temperatures (T@sub m@'s) than the solution phase, but also show discrimination between matched and mismatched sequences under low stringency conditions. The orientation of the probe is highly dependent on the method of immobilization to the surface. Non-optimal attachment can orient the probe in a way that interferes with duplex formation, or such that it becomes buried within the surface and inaccessible to the target sequence. The substrate used for array preparation ultimately controls both density and orientation; optimization of the substrate can enhance array performance in an assay. Especially interesting is a precise control of probe density, where the density effects alter the T@sub m@'s of immobilized sequences to allow a broad range of sequence to be analyzed in a single temperature assay. We have been exploring a variety of silane modified substrates for the preparation of oligonucleotide arrays, focusing on immobilization of the probe through amine or thiol moieties. The effect of density and attachment chemistry on target adsorption and single-base mismatch discrimination with single and double stranded oligonucleotide target sequences will be presented.

2:40pm **BI+NS-ThA3 Investigation of DNA Hybridization on Surfaces by Surface Plasmon Fluorescence Spectroscopy (SPFS)**, ***T. Neumann***, *W. Knoll,* Max-Planck Institute for Polymer Research, Germany

The investigation of DNA hybridization on surfaces, and as a consequence, the development of DNA-chips and sensors, has been of increasing interest in recent years, since such technology can be used to investigate the human genome. We report here a study of PNA:DNA and DNA:DNA hybridization on surfaces measured by surface plasmon resonance spectroscopy (SPS) coupled with fluorescence (SPFS) and surface plasmon microscopy (SPM). PNA (peptide nucleic acids) and DNA catchers were immobilized either directly on gold surfaces by thiol linkers or via biotin on streptavidin covered gold surfaces. In order to enhance the detection limit of the SPR, the fluorescence signal of dyes attached to the target strands was detected during the hybridization to the immobilized catchers. To get a deeper insight into the underlying principles of the hybridization process near surfaces, conformational changes in the catcher and target DNA structure were monitored by comparing the hybridization kinetics obtained by having the fluorescent dye attached either to the catcher or the target and varying the length of both types of strands. Furthermore by using two different fluorescent dyes on the DNA strands, we were able to carry out Forster transfer experiments during the hybridization step, which allowed us to monitor distance changes between the catcher and target at the surface.

3:00pm **BI+NS-ThA4 Fluorescence Detection of Surface DNA Hybridization Reactions Based on Surface Structural Changes**, ***T.H. Huang***, *S.J. Stranick, M.J. Tarlov,* National Institute of Standards and Technology

We describe a novel fluorescence method for the detection of surface-immobilized DNA hybridization reactions. Solid phase hybridization reactions form the basis of DNA chip technologies that are used for sequencing and genetic diagnostic applications. In conventional fluorescence-based detection schemes, the "target" DNA is typically labeled with a fluorophore. We report a method where instead, the "probe" DNA is labeled with a fluorophore. Our model surface hybridization system uses a mixed monolayer of fluorescein-tagged, thiol-derivatized, single-stranded DNA probes and 6-mercaptohexanol self-assembled on Au surfaces. Prior to hybridization, the fluorophore on the probe is in closer proximity to the gold surface, resulting in greater quenching of the fluorescence signal. Upon hybridization, the double-stranded DNA adopts a rod-like structure that extends the fluorophore away from the gold surface. With the fluorphores located further from the gold surface, quenching is reduced and an increase in fluorescence intensity is observed. Parameters affecting fluorescence intensity such as probe surface coverage, probe length, and target concentration will be discussed. In addition, a comparison of probe- and target-labeled fluorescence detection schemes will be made.

3:20pm **BI+NS-ThA5 Silicon Surface Chemistry for DNA Immobilization**, *T. Strother, Z. Lin, W. Cai, L.M. Smith,* ***R.J. Hamers***, University of Wisconsin, Madison

Many emerging areas of biotechnology, such as "gene chips", seek to leverage many aspects of the existing infrastructure in microelectronics and apply it to new areas. While most previous work has focused on the attachment of DNA to surfaces of gold or glass, we have investigated the chemistry involved in covalently bonding DNA to Si(001) and Si(111) surfaces in a way that retains its ability to selectively hybridize with its solution-phase complements. The use of XPS to study the chemical structure at each step in the DNA attachment process has lead to the development of new procedures that are both simple and robust. Starting with hydrogen-terminated Si(001) and Si(111) surfaces, photochemical excitation at 254 is used to remove the photo-labile hydrogen; the exposed surface is then reactive toward organic molecules containing one or more unsaturated C=C bonds. "Linker" molecules containing a C=C double bond with another functional group(such as an amine or ester group) are then used to provide a dense set of surface-bound functional groups for attachment of DNA. To control the selectivity of the attachment process, however, careful optimization of the molecular structure of the linker and the other processing conditions are required. The density and chemical uniformity of the surface (as judged by XPS) is highly correlated with the intensity and selectivity achieved in subsequent binding of the surface-bound DNA to its fluorescently-labeled complements in solution. The results show that control of surface chemistry indeeds leads to significant improvements in the formation of DNA-functionalized silicon surfaces.

3:40pm **BI+NS-ThA6 BARC: A Magnetoresistive Biosensor@footnote 1@**, ***P.E. Sheehan***, Naval Research Laboratory*; R.L. Edelstein,* Geo-Centers, Inc.*; C.R. Tamanaha, M. Miller,* Naval Research Laboratory*; L. Zhong,* Geo-Centers, Inc.*; R.J. Colton, L.J. Whitman,* Naval Research Laboratory

The Bead ARray Counter (BARC) is a revolutionary biosensor that uses DNA microarrays, magnetic microbeads, and giant magnetoresistive (GMR) magnetic field sensors to detect and identify biological molecules.@footnote 2@ The current prototype is a table-top instrument with integrated fluidics under development for the detection of biological warfare agents. The core of the sensor is a small, microfabricated chip containing a GMR sensor array for detection of up to eight different pathogens. Oligonucleotide probes complementary to pathogen target sequences are arrayed onto the microfabricated chip directly above the GMR sensors. Specific hybridization is measured and discriminated from non-specific background by addition of functionalized magnetic microbeads that bind to the captured target DNA. The beads tethered to the surface are detected by the GMR sensors, with the intensity and location of the signal indicating the concentration and identity of the target pathogens. A complete assay, including hybridization and detection can be performed in approximately 30 min. Because each GMR sensor is capable of detecting a single magnetic bead, in theory, the BARC biosensor should be capable of detecting a single molecule. With recent advances in GMR technology for computer memory, chips with millions of sensors will soon be commercially available, enabling the development of a BARC sensor capable of detecting thousands of analytes simultaneously. We will discuss the scientific and technical challenges to making such a sensor system a reality. @FootnoteText@ @footnote 1@Supported by the Defense Advanced Research Projects Agency. @footnote 2@Edelstein et al., Biosensors & Bioelectronics 14, 805 (2000).

4:00pm **BI+NS-ThA7 A Biosensor Based on Force Differentiation@footnote 1@**, ***C. Yanavich***, *M. Malito,* Nova Research, Inc.*; G.U. Lee, L.J. Whitman, R.J. Colton,* Naval Research Laboratory*; M. Natesan,* Geo-Centers, Inc.

We are developing an array biosensor that uses a magnetic force to differentiate specific ligand-receptor binding from non-specific ligand-surface binding. In this force differentiation assay biosensor, capture antibodies that will bind to specific target analytes within a sample are first

coated onto a surface. The captured analyte is then sandwiched by a second antibody that is attached to a magnetic microbead. A magnetic force with well-defined magnitude and orientation is applied to remove the non-specifically adsorbed beads, and a semi-automated optical reader then measures the number of the specifically bound beads remaining on the surface (which can be correlated with the analyte concentration). The original prototype used polystyrene microtitre wells for multi-analyte detection. We are now developing a second-generation sensor that employs a filter membrane as the capture surface. The filter facilitates concentration of the antigen on the surface and enhances the antigen-antibody interactions, significantly reducing the assay time (to ~30 min), and increasing the sensitivity by two-to-three orders of magnitude. Several techniques are being investigated to pattern capture antibodies onto the filter surface in order to enable multi-analyte detection on a single disposable filter. They include photo patterning with photo-activated biotin or caged photo-biotin, and imprinting via PDMS masks. We will also discuss our development of alternate techniques aimed at simplifying the bead counting system. @FootnoteText@ @footnote 1@Supported by the Joint Service Technical Panel for Chemical and Biological Defense (JSTPCBD).

4:20pm **BI+NS-ThA8 Encapsulation of Smart Polymers in Silica: Stimuli-Responsive Porous Hybrid Materials That Incorporate Molecular Nano-Valves**, *G.V. Rama Rao,* ***G.P. Lopez,*** University of New Mexico*; A. Chilkoti,* Duke University

Elastin (a protein) and poly(N-isopropyl acrylamide) (PNIPAAM, a synthetic polymer) are two types of thermo-sensitive, smart polymers which exhibit inverse solubility in water upon heating and undergo the transition from hydrophilic conformations to hydr ophobic conformations at a temperature known as lower critical solution temperature (LCST). This interesting property has led to have several applications in biotechnological areas. In this report, we describe the development of molecular switches as nan os copic actuators that can control the transport of chemical species by encapsulating PNIPAAM and elastin in dense silica gels by sol-gel synthesis. Cycling through the LCST can control molecular permeability through these hybrid materials. The pores res ult ing from the transition can selectively transport molecular species depending on their size. For example, permeation experiments revealed the LCST behavior of PNIPAAM in silica-polymer membranes and was identified to be 31 °C. DSC studies on bu lk gels are in good agreement with the permeation results. Cycling of the membranes between 25 and 40 °C indicates the membranes possess reversible, variable permeability while maintaining good mechanical stability. Importantly, permeation experiments on PNIPAAM-si lica membranes with various molecular weights of poly(ethylene glycol) have clearly demonstrated that the membrane is acting as a molecular switch by being impermeable below the LCST, and permeating the lower molecular weights of poly(ethylene glycol) and filtering out higher molecular weight polymers above LCST.

4:40pm **BI+NS-ThA9 Adsorption Behavior and Optical Properties of Surface-Adsorbed Polystyrene Nano Particles**, ***M. Himmelhaus,*** Universität Heidelberg, Germany*; H. Takei,* Hitachi Central Research Laboratory, Japan

Polystyrene (PS) nano particles have become popular tools in photonics, nano technology, and life science since they have become commercially available in a wide range of sizes with narrow size distribution. While most applications utilizing surface-adsorbed PS nano particles deal with ordered arrays on mesoscopic scale, recently a chemically induced method for adsorption of such particles was introduced to yield random-close-packed (rcp) monolayers of almost arbitrary lateral extension. Such layers can be used as a template for the formation of cap-shaped Gold nano particles that exhibit extraordinary optical properties@footnote 1@ and thus can be developed into a sensitive optical biosensor.@footnote 2@ Here we demonstrate that the chemically induced adsorption method can be combined with alkanethiol chemistry to gain better control of sphere adsorption. Thus, sphere layers of varying density can be fabricated and their optical properties can be studied as a function of coverage. By further utilizing Micro Contact Printing (µCP) of tailgroup modified alkanethiols 2D patterns of rcp PS sphere monolayers with a lateral resolution of a few microns and a total pattern area of ~1 cm@super 2@ can be produced. These patterns are a first step to the development of an optical biosensor based on cap-shaped Gold nano particles with massively parallel detection capability. @FootnoteText@ @footnote 1@ H. Takei, J. Vac. Sci. Technol. B 17 (5) 1906, 1999 @footnote 2@ M. Himmelhaus, H. Takei, Sens. Acuators B 63 (1-2) 24, 2000

5:00pm **BI+NS-ThA10 Formation and Patterning of Supported Fluid Lipid Bilayers on a High Refractive Index Substrate**, ***C.M. Ajo,*** *L.C. Kam, S.G. Boxer,* Stanford University

Supported lipid bilayers are a useful model system to probe cellular membrane components and their interactions in a near native environment. Specifically, membrane components reconstituted in supported lipid bilayers create a well-defined two-dimensional system that can be manipulated - and then interrogated with a variety of surface specific and optical techniques. Several of these techniques rely on evanescent fields to probe the region near the solid support-lipid bilayer interface. However, the solid support typically has been a low refractive index material that permits the evanescent wave to penetrate significantly beyond the bilayer (650 Å). Here we report the formation of supported lipid bilayers on lithium niobate (LiNbO@sub 3@), a material with a high refractive index (n=2.3). Vesicle fusion onto lithium niobate forms a single uniform supported lipid bilayer that exhibits lateral diffusion properties similar to glass-supported lipid bilayers. By blotting and stamping,@footnote 1@ supported bilayers can be patterned reversibly, and the lipid components reorganize in response to an electric field. The high refractive index of lithium niobate restricts the penetration of an evanescent field to within 160 Å of the solid support-lipid bilayer interface. This provides a method to study the cell-supported lipid bilayer interface, since the relevant distances are on this order. Additionally, the transparency of lithium niobate ove a wide range of wavelengths makes it a useful substrate for both visible and infrared studies. @FootnoteText@ @footnote 1@J. S. Hovis and S. G. Boxer, Langmuir 16, 894 (2000).

Biomaterial Interfaces
Room 202 - Session BI-FrM

Biomolecular Recognition at Surfaces

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

8:20am **BI-FrM1 AFM and EM Studies Providing Insights into Membrane Fusion in Cells**, ***B.P. Jena,*** Yale University School of Medicine **INVITED**

Binding force profiles between solubilized synaptic vesicle and synaptosomal membrane components were examined using atomic force microscopy (AFM). These AFM force spectroscopic studies reveal that a 17 nm and a 34 nm long complex form, following interaction between the two sets of membrane components. The formation of such complexes is further confirmed using negative staining electron microscopy (EM), performed on the immunoprecipitated membrane fusion machinery obtained from neuronal tissue. These EM studies performed on the dehydrated immunoprecipitated native fusion complex reveal the presence of 28-30 nm new coiled rod-like structures in association with 14 nm long SNARE complexes. Neuronal SNAREs were found coiled and super-coiled with these structures. The existence of NSF as pentamers in its native state is also demonstrated. The extent of coiling and super-coiling of SNAREs may regulate the potency and efficacy of membrane fusion in cells.

9:00am **BI-FrM3 Adhesion of Mammalian Cells Modeled by Functionalized Vesicles**, ***A. Janshoff,*** *J. Wegener, H.-J. Galla,* WWU Muenster, Germany

Specific molecular recognition between cell-surface receptors and extracellular matrix proteins immobilized on a growth substrate are the most relevant interactions that allow cells to actively spread on a surface. We applied the quartz crystal microbalance technique (QCM) to follow the time course of cell attachment and spreading on artificial substrates. The shift of the sensorÂ´s resonance frequency provides a direct measure for the fractional surface coverage of the piezoelectrically active area. Frequency shifts associated with the establishment of confluent cell layers were found to be dependent on the cell species, reflecting their individual impact on the displacement of the resonator. In order to learn more about the mechanisms that govern the response of shear wave resonators to the attachment of mammalian cells, we modeled the cellular system with unilamellar liposomes doped with biotinylated lipids. Liposome adhesion to avidin/streptavidin coated surfaces was monitored using the QCM technique. Increasing fractions of the biotinylated lipid in the liposome shell resulted in enhanced shifts of the resonance frequency. Concomitant shape analysis of the surface-attached vesicles using SFM revealed an extended contact area between liposome and surface. We conclude, that an increasing number of bonds between the liposome and the surface induces the extended contact areas and that a similar mechanism may be applicable to explain the individual response of the QCM to different cell species.

9:20am **BI-FrM4 PNA-DNA and DNA-DNA Hybridization Detection via Lipid-Biotin-Streptavidin Immobilization on a SiO2 Coated Quartz Crystal Microbalance Sensor**, ***F. Höök,*** *A. Ray, B. Norden, B. Kasemo,* Chalmers University of Technology, Sweden

Surface-based bioanalytical sensors for oligonucleotide hybridization are very attractive for genetic diagnostics, gene therapeutics and the emerging solid phase / real time PCR. Little is however known about how various immobilization strategies affect the conformation and hence function of oligonucleotide strands. We have investigated the possibility to use the dissipative quartz crystal microbalance (QCM-D) technique and controlled surface-immobilization of single stranded synthetic peptide nucleic acid (PNA) as well as DNA, as selective probe(s) for fully complementary and various single mismatch DNA. In order to minimize unspecific binding of DNA, streptavidin was immobilized as a protein 2-D crystal on a biotinylated phospholipid bilayer supported on a SiO@sub 2@ surface in the fluid liquid crystalline phase. This was followed by attachment of a mixed-sequence 15-mer biotin-PNA or a 15-mer biotin-DNA with identical base pairs. The exposure of the streptavidin-immobilized biotin-PNA and biotin-DNA to fully complementary and various mismatch DNA was investigated at 24 °C. Only the fully complementary and singly mismatched DNA oligomers hybridized with the immobilized PNA and DNA, and was possible to discriminate via significant difference in the binding and dissociation kinetics, demonstrating a very high selectively. Most interestingly, however, is that the ratio between hybridization-induced energy dissipation (c.f. rigidity) and the frequency shifts (c.f. mass uptake), allowed us to discriminate different structures of immobilized PNA-DNA and DNA-DNA duplexes. Possible influence on the hybridization kinetics and the structure of the formed duplexes from primarily lateral interaction is discussed.

9:40am **BI-FrM5 DNA Probe Structure and Target Length Effects on Hybridization Kinetics and Efficiency of DNA Self-assembled Monolayers**, ***G.B. Saupe,*** *M.J. Tarlov,* National Institute of Standards and Technology

Optimizing the parameters involved in monolayer DNA hybridization events is important to the emerging DNA sensor array technologies used for a variety of applications including genetic diagnostics, forensics, and infectious disease detection. The objective of our research is to determine how DNA surface coverage, molecular orientation, and sequence identity impact the functionality of DNA array devices. To study these factors we use a model system with short sequences of single-stranded DNA probes self-assembled on gold surfaces through a thiol linker. The gold is also passivated with mercaptohexanol to eliminate non-specific adsorption of DNA to the gold and to enhance the activity of immobilized probes. Surface Plasmon Resonance is used to monitor and derive, in situ, the nanometer-scale thickness changes associated with surface hybridization reactions. Complementary single-stranded DNA targets in high salt buffered solutions hybridize with relatively high efficiency (25-100%) to these surface bound probes. We will report how variations in probe surface structure, the length of ssDNA targets, and the relative position of the complementary sequence in the ssDNA targets affect hybridization kinetics, efficiencies and completion times.

10:00am **BI-FrM6 Probing Protein Interactions with Surface-Immobilized Double-Stranded DNA Using Surface Plasmon Resonance Sensing Techniques**, ***J.S. Shumaker-Parry,*** *C.T. Campbell, K.E. Nelson,* University of Washington*; G.D. Stormo,* Washington University Medical School*; F.S. Silbaq,* University of Colorado*; R.H. Aebersold,* University of Washington

Understanding the molecular mechanisms of gene expression in eukaryotes requires a precise knowledge of the strength and specificity of protein:DNA interactions. Surface plasmon resonance sensing techniques are important for monitoring the adsorption of biomolecules from liquid solutions onto functionalized solid surfaces with high sensitivity and fast time response. Simple methods convert changes in the angle or wavelength at which the surface plasmon resonance (SPR) of a thin metal film is optically excited into adsorbate concentrations. Methods for monitoring interactions between the transcription repressor protein Mnt and surface-immobilized double-stranded DNA using SPR spectroscopy and microscopy will be described. We have immobilized dsDNAs onto a planar gold surface with high density (1-3x10@super 12@ DNA/cm@super 2@, depending on their length) and uniform spacing (~4 nm closest possible DNA-DNA separation). This was accomplished by adsorbing biotinylated DNAs onto a nearly close-packed monolayer of the protein streptavidin prepared first by adsorbing it onto a mixed self-assembled monolayer on gold containing biotin-terminated and oligo(ethylene glycol)-terminated alkylthiolates in a 3/7 ratio. This DNA-functionalized surface resists non-specific protein adsorption. SPR spectroscopy experiments have shown that Mnt binds in 3.8:1 ratio to its immobilized DNA recognition sequence. This is consistent with its behavior in homogeneous solution, where it binds as a tetramer to its DNA binding sequence. A sequence with a single base-pair mutation shows nearly as much Mnt binding, but a completely random DNA sequence shows only 5% of this binding. This proves that DNA-binding proteins bind sequence-specifically to dsDNAs that are immobilized to gold with this streptavidin linker layer. SPR microscopy is being developed to extend these studies to probe protein interactions with an array of dsDNA-containing elements immobilized on a sensor surface.

10:20am **BI-FrM7 Immobilized Antibodies on Functionalized Self-assembled Monolayers: Reactivity, Surface Homogeneity and Microarraying**, ***P. Kernen,*** *F. Zaugg, K. Witte, D. Quincy, P. Indermuehle, S. Nock, P. Wagner,* Zyomyx Inc.

@omega@-Substituted alkanethiols self-assemble in ordered monolayers on Au(111) surfaces and form highly reproducible reactive interfaces for biomolecule immobilization. Reaction conditions for covalent coupling of antibodies to monolayers exposing N-hydroxysuccinimide and other functionalities have been tested using radiometry and fluorescence microscopy. Scanning probe microscopy, X-ray photoelectron spectroscopy and other surface analytical techniques have been applied to characterize homogeneity and surface coverage of covalently bound biomolecules. By incorporating these bioreactive interfaces into microfabricated three-dimensional structures in silicon, functional microarrays of antibodies could be produced with variable feature size.

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