Biomaterial Interfaces Division
Room: 108 - Session BI-MoM

Biomolecules at Interfaces

Moderator: A. Rosenhahn, Karlsruhe Institute of Technology, Germany

8:20am **BI-MoM1 Cell Instructive Biomaterials by Non-Equilibrium Self-Assembly**, *R.V. Uljin, V. Jayawarna, S. Roy, M.J. Dalby,* The University of Strathclyde, UK, ***S.L. McArthur***, Swinburne University of Technology, Australia **INVITED**

Peptides are highly versatile building blocks for the production of supramolecular gels which mimick certain properties of biological systems but with minimal complexity. These gels may be ideally suited to interface synthetic systems with biology, and as such have attracted much interest in a range of areas including health care and energy related technologies. We have developed versatile gelator systems based on aromatic peptide amphiphiles, which self-assemble via a unique mechanism involving π-interlocked β-sheets. These systems display highly tunable supramolecular functionalities, giving rise to materials with controlled mechanical properties, nanotopography and bioactive properties. We will show that these properties can be controlled by exploiting non-equilibrium self-assembly which may involve the use of biocatalysts to control the self-assembly process. We will discuss progress in fundamental understanding of these systems as well as applications as matrices for stem cell differentiation.

References:

Nature Chemistry, 2010, 2, 1089-1094..; Chem. Soc. Rev. 2010, 39, 3351-3357.; Nanoscale, 2010, 2, 960-966.; Chemical Communications, 2010, 46, 3481-3483.; Nature Nanotechnology, 2009, 4, 19-24.; Langmuir, 2009, 25, 9447-9453.; Faraday Discussions, 2009, 143, 293-303.; Langmuir, 2009, 25, 7533–7539.; Acta Biomaterialia, 2009, 5, 934-943.; Biomaterials, 2009, 30, 2523-2530.; Soft Matter, 2009, 5, 1728-1734.; Small, 2009, 5, 587-590.; Adv. Mater., 2008, 20, 37-41.; Small, 2008, 4, 279-287.

9:00am **BI-MoM3 Lipid Vesicle Fusion for Studies of Cell Functions**, ***L. Simonsson***, *A. Gunnarsson, M. Kurczy, P. Jönsson, AS. Cans, F. Höök*, Chalmers University of Technology, Sweden

Fusion of lipid vesicles and cells is a natural process which takes place in eukaryotic cells. It is a vital process, since it enables cells' communication with the outside, both via vesicle content release and through delivery of e.g. membrane proteins to the outer cell membrane. Exocytosis is still not fully understood and although attempts have been done to deliver membrane constituents to supported lipid bilayers, improvement in e.g. efficiency remains. In order to gain a deeper understanding of the mechanism of membrane fusion as well as improve the delivery of arbitrary membrane constituents including complete cell membrane fragments, to supported lipid bilayers, we have in this work developed two novel and powerful techniques.

To mimic exocytosis, we use giant unilamellar vesicles (GUVs) as a model of the cell membrane, cholesterol-DNA[1-3] as a mimic of the SNARE-proteins and small unilamellar lipid vesicles filled with easily oxidized catechol to represent the cellular vesicles. We probe the fusion process using a carbon fiber electrode, detecting the released catechol. By building this advanced but yet controllable model system of exocytosis, we believe that a wide range of studies can be made in order to decipher the process of exocytosis. Future applications are delivery of e.g. membrane proteins to GUVs, as well as for vesicular drug delivery to cells.

In order to deliver membrane constituents to SLBs, we use a controlled bulk flow through a microfluidic channel to move the front edge of a supported lipid bilayer and fuse it with vesicles adsorbed in front of it. The membrane constituents of the adsorbed vesicles are efficiently incorporated into the supported lipid bilayer and can be manipulated in 2D (accumulated and separated) by again using the bulk flow. We show that this method is perfectly compatible with cell membrane fragments derived directly from 3T3 fibroblast cells[4]. The method enables studies of e.g. receptor-ligand interactions as well as membrane protein separation in a native environment.

Simonsson et al., ChemPhysChem, (2010)

Stengel et al., J. Am. Chem. Soc., (2007)

Stengel et al. J. Phys. Chem. B, (2008)

Simonsson et al., Submitted

9:20am **BI-MoM4 Watching Biomineralization at Work: The Specific Interactions of Statherin with Hydroxyapatite Surfaces Probed at the Molecular Level**, ***T. Weidner***, *M. Dubey, K. Li, J. Ash, J.E. Baio,* University of Washington, *C. Jaye, D.A. Fischer,* National Institute of Standards and Technology, *G.P. Drobny, D.G. Castner*, University of Washington

Biomineralization proteins act as nature's crystal engineers and adsorb onto crystal surfaces with high binding affinity and precision using specific substrate-surface binding motifs. Owing to the importance of the underlying physiological processes and a general interest in biomineralization mechanisms, the binding of regulatory proteins has attracted significant interest. We have studied statherin, which regulates the growth of hydroxyapatite (HAP) in bone and tooth enamel and prevents the buildup of excess HAP by inhibiting spontaneous calcium phosphate growth. A detailed understanding of the underlying molecular recognition mechanisms would help bioengineers and scientists to devise new biomimetic approaches for clinical applications and biomineral nanofabrication. Sum frequency generation (SFG) spectroscopy can probe protein orientation and secondary structure at the solid-liquid interface and we have recently shown it can address specific protein regions with atomic resolution when combined with isotopic labeling and solid state NMR (ssNMR) data.(1) We have combined both techniques with near edge X-ray absorption fine structure (NEXAFS) spectroscopy to characterize the structure of the binding domain of statherin, SN-15, on HAP. Protein adsorption was verified using XPS and ToF-SIMS. NEXAFS N *K*-edge spectra clearly show that hydrogen bonding is important for the binding of both peptides. SFG confirmed an α-helical secondary structure of SN-15 on HAP with the helix axis parallel to the surface. Deuteration was used to specifically probe the orientations of all hydrophobic side chains (leucine, isoleucine, phenylalanine) with SFG *in situ*. The leucine chain was tilted 120° from the surface normal (pointing towards the surface) and isoleucine was tilted 5° from the surface normal. We also employed fluorine labels to probe individual phenylalanine rings with NEXAFS spectroscopy. Measurements of ring orientations in combination with ssNMR surface distance and rotamer dynamics data allowed us to develop a clear picture of the side chain structure.

1. Weidner T, Breen NF, Li K, Drobny GP, & Castner DG (2010) A Sum Frequency Generation and Solid-State NMR Study of the Structure, Orientation and Dynamics of Polystyrene-Adsorbed Peptides. *Proc. Natl. Acad. Sci. U. S. A.* 107:13288–13293.

9:40am **BI-MoM5 ECM Ordering Effects as a Marker for Early Tissue Formation on Artificial Substrates - A Sum-Frequency-Generation Spectroscopy Study**, *M.-O. Diesner,* ***P. Koelsch***, Karlsruhe Institute of Technology (KIT), University of Heidelberg, Germany

The *in situ* monitoring of the interphase between a substrate and a cellular layer is of great interest as it allows determination of changes in surface properties and extracellular matrix (ECM) organization. The latter is an early indicator of major cellular processes like migration, adhesion, proliferation, metastasis, tissue formation, and gain or loss of differentiation occurring. Typically, ECM studies of adherent cells involve labeling and fixing cell samples, which may result in their disruption and in the loss of valuable information. In addition, the weak signal-to-noise ratio of fluorescent probes limits the probing capabilities at early stages of cell adhesion.

Recent work from our group has shown that sum-frequency-generation (SFG) spectroscopy can be used to interrogate the ordering of the ECM beneath adherent cells on an artificial substrate during these early stages.[1,2] It turns out that SFG spectroscopy is suitable to probe the layer in between a solid substrate and living cells and that the information which can be obtained on such systems are twofold: first, changes of the surface coating can be investigated in real-time and in vitro on a molecular scale while cells adhere to it. Secondly, SFG spectroscopy is suitable for the determination of ordering parameters within the ECM without the need for labeling and processing.

In this contribution we will report on ordering phenomena occurring at early stages of rat and mouse embryonic fibroblasts adhesion on Au-coated Si wafers. Several phases observed during the adhesion process will be discussed and the results obtained by nonlinear optical spectroscopy will be correlated to classical tools including Western blot analysis of ECM constituents, fluorescent probes, and genetic screens blocking the formation of fibrils.

References

[1] M.-O. Diesner, C. Howell, V. Kurz, D. Verreault, and P. Koelsch. In vitro characterization of surface properties through living cells. J Phys Chem Lett, 1:2339– 2342, 2010.

[2] C. Howell, M.-O. Diesner, M. Grunze, and P. Koelsch. Probing the extracellular matrix with sum-frequency-generation spectroscopy. Langmuir, 24:13819–13821, 2008.

10:00am **BI-MoM6 Structure and Function of von Willebrand Factor on Synthetic Surfaces and Collagen**, ***E. Hillenmeyer***, *O. Yakovenko, R. Penkala, W. Thomas, D.G. Castner*, University of Washington

von Willebrand Factor (VWF) is a soluble clotting protein responsible for binding platelets through the glycoprotein 1b platelet receptor. VWF can become activated and bind platelets when bound to collagen in an injured blood vessel or under increased shear.

VWF can also bind platelets when adsorbed to synthetic surfaces, specifically biomaterials. There is evidence that surface characteristics influence VWF adsorption. Previous AFM studies of VWF adsorbed to hydrophilic (mica) and hydrophobic (octadecyltrichlorosilane modified glass) surfaces showed differences in adsorbed topography[1]. However, studies were not performed to relate adsorption differences to VWF function.

Previous studies in our lab have shown differences in function and structure of the platelet binding domain of VWF (A1 domain) when adsorbed to three surfaces: polystyrene (PS), tissue culture polystyrene (TCPS), and glass, with A1 most active when adsorbed onto PS. A1 function was tested by measuring platelet binding under flow. A1 surface structure was investigated using time of flight secondary ion mass spectrometry (ToF-SIMS) and binding of conformation-dependent antibodies in ELISA assays.

In the work presented here, we have used surface analysis techniques to obtain greater detail about structural differences of the VWF A1 domain adsorbed onto synthetic surfaces. We used near-edge x-ray adsorption fine structure (NEXAFS) to examine the amide backbone, corresponding to the pi* feature of the nitrogen edge NEXAFS spectrum. Differences were observed in the angle dependence of the spectra when A1 was adsorbed onto PS, TCPS, and glass, indicating significant structural differences in the protein when adsorbed onto different surfaces. Sum Frequency Generation (SFG) was also used to probe the structure of the amide backbone using amide I spectra.

In addition to examining VWF on synthetic surfaces, we have used ToF-SIMS to obtain structural information about A1 bound to collagen, as occurs during *in vivo* injury. Principal component analysis of ToF-SIMS data showed differences between A1 bound to collagen and A1 adsorbed directly onto polystyrene. This suggests that A1 adopts different conformations on the natural versus synthetic substrates, potentially leading to different mechanisms of activation.

In this work, we use surface analysis tools to increase our understanding of VWF behavior, both on synthetic surfaces and in complex, layered protein systems. Increasing our fundamental knowledge of VWF can improve our understanding of VWF interactions with biomaterial surfaces, as well as thrombosis during injury.

1. M. Raghavachari, et al. *Colloids Surf B* (2000) 19:315.

10:40am **BI-MoM8 ToF-SIMS Study of Orientation of $FnIII_{9-10}$ Fibronectin Fragment on Self-Assembled Monolayers**, ***L. Árnadóttir***, *L.J. Gamble*, University of Washington

Fibronectin (FN) is an extra cellular matrix protein that is involved in many cell processes such as adhesion, migration and growth. The orientation and conformation of FN adsorbed onto surfaces can therefore have a critical effect on cell-surface interactions. Experimental orientation studies of model systems also provide an important benchmark for molecular simulations and are of great value for further development of force fields used in many theoretical studies of protein adsorption. In this study the adsorbed orientation of the 9-10 fragment of FNIII was studied on three different model surfaces (self-assembled monolayers (SAM) of C_{11} alkanethiols on Au, $-CH_3$, $-NH_2$, and -COOH terminated SAMs.) The $FNIII_{9-10}$ fragment includes the two binding sites for the FN protein, a RGD site on segment 10 (seg10) and a synergy site on segment 9 (seg9). This fragment also has a significant asymmetry in its amino acid distribution with His found exclusively on seg9, Lys only on seg10 and three times more Tyr on seg10 then seg9. Taking advantage of this asymmetry, we use time of flight secondary ion mass spectrometry (ToF-SIMS) to study the different orientation on different surfaces and X-ray photoelectron spectroscopy (XPS) to determine the difference in surface coverage. While CH_3 and NH_2 have similar full coverage the highest COOH coverage is about half the maximal coverage observed for the two other surfaces. A ToF-SIMS comparison of the $FNIII_{9-10}$ on COOH and NH_2 at similar coverages shows significantly more His on the COOH and more Tyr on the NH_2 surfaces suggesting an opposite orientation of the fragment on these two surfaces. Results indicate that on the COOH surface the fragment is oriented with seg10 down while on the NH_2 surface seg9 is closer to the surface. Comparing similar coverages of the $FNIII_{9-10}$ on NH_2 and CH_3, the later has less Tyr signal (from seg10) suggesting that the seg10 is closer to the surface on CH_3 than on NH_2. In contrast, when $FNIII_{9-10}$ on CH_3 and COOH results were compared, the fragment on the CH_3 sample has less His signal suggesting that seg9 is closer to the surface compared to COOH.

11:00am **BI-MoM9 Single Protein Manipulation with STM**, ***S. Khan***, *K. Clark, C. Henneken, E. Rauh, S.-W. Hla*, Ohio University

Scanning tunneling microscope (STM) is not only an instrument to image atomic landscape of material surfaces but also is a tool to manipulate individual atoms and molecules. If STM manipulation and spectroscopy can be applied to individual biomolecules, it will be advantageous for multiple research areas. Here we use a low temperature STM in an ultrahigh environment to image individual protein molecules with molecular resolution on Ag (111) surface at 4.6 K. A-b type amyloid precursor proteins molecules and various single chain proteins were deposited on atomically clean Ag (111) surface. STM images and tunneling spectroscopy enables direct sequencing of amino acid groups in these molecules. Moreover, using an STM manipulation procedure employing an attractive tip-molecule interaction, individual molecules were relocated on the surface; thereby their mechanical integrity can be tested. Protein folding is a major issue in biological processes. Here, we will demonstrate that folding of carbon back-bone in these proteins is possible using an STM manipulation procedure. This experiment presents a novel avenue of biological research where sequencing and manipulation may be performed one molecule-at-a-time. We acknowledge the financial support provided by US-DOE-DE-FG02-02ER46012.

11:20am **BI-MoM10 DNA Origami from Inkjet Synthesis Produced Strands**, ***I. Saaem***, *A. Marchi, J. Tian, T. LaBean*, Duke University

High-throughput synthesis of quality mixed oligonucleotides (oligos) allows for exhaustive studies of DNA nanostructured material formation. In situ DNA synthesis was achieved with a custom piezoelectric inkjet system using phosphoramidite chemistry, on functionalized cyclic olefin copolymer (COC) as a chip substrate. When amplified off of the chip via strand displacement amplification (SDA), the oligos were eluted and used directly for constructing multiple DNA origami nanostructures in a one-pot assembly. By performing SDA, each chip may be reused multiple times to produce pools of staple strands for repeated experiments. Two separate DNA origami structures were formed from a double-stranded scaffold strand. By redesigning specific staple strands, these structures can be connected to provide a larger nanostructured template.

11:40am **BI-MoM11 Solid State Electron Transport across Proteins**, ***D. Cahen***, *M. Sheves, I. Pecht, L. Sepunaru*, Weizmann Institute of Science, Israel

We found that solid-state electron transport (Etp) studies are possible across non-modified 'dry' proteins, between two solid electrodes, using the light-induced proton pumping protein, Bacteriorhodopsin (bR), the electron transfer (ET) protein, Azurin (Az), and Bovine Serum Albumin (BSA). Clear differences between these proteins, where for bR and Az we can show that they preserve their structure in the solid state measurement configuration, were observed, with small tunneling decay constants for all three proteins, suggesting that incoherent transport is the dominant mechanism. Putting our data in perspective by comparing them to all known protein Etp data in the literature, we concluded that, in general, proteins behave more akin to molecular wires than to insulators. An important part of these studies was the modification of the proteins by, e.g., removing or disconnecting the retinal in bR and removing the Cu redox centre in Az.

We now report that, notwithstanding the above-noted earlier conclusion on hopping, which was based on the Etp efficiency, Az shows 9-360K temperature independent Etp, until its denaturation temperature. Removal of the Cu changes this behavior to Arrhenius-like thermally activated Etp, which becomes temperature independent below ~ 200K, a behavior that apo-Az shares, qualitatively with bR, its variants and with BSA.

This difference between bR and Az leads to the truly remarkable situation that above room temperature the non-ET protein bR shows significantly better Etp than ET protein Az.

Biomaterial Interfaces Division
Room: 108 - Session BI-MoA

Sensors and Fluidics for Biomedical Applications
Moderator: E. Reimhult, University of Natural Resources and Life Sciences, Austria

2:00pm **BI-MoA1 Three-dimensional Microfluidic Flow Cell Array Integrated with SPR Microscopy for Multi-channel Bioassays**, ***J.S. Shumaker-Perry***, University of Utah **INVITED**

The expanding development and implementation of biotherapeutics to treat a wide range of diseases, including autoimmune diseases and cancer, have increased the need for immunogenicity assessment of these therapies. Immunogenicity is an immune system response of a patient to a drug. Anti-drug antibodies (ADAs) produced during the immune response may cause serious adverse effects such as reducing drug efficacy, altering pharmacokinetics, causing infusion reactions including anaphylaxis and serum sickness, and neutralizing native proteins. The complexity of immunogenicity analysis presents challenges in identifying and characterizing the neutralizing activity of ADAs. We have integrated a three-dimensional microfluidic flow cell array (MFCA) with surface plasmon resonance microscopy (SPRM) for a multi-channel, array-based approach to immunogenicity assessment. The microfluidic device provides 48 separate flow channels that can be used simultaneously for biomolecule immobilization and subsequent array-based biomolecule interaction analysis. Because the biomolecules can be immobilized *in situ*, exposure to harsh environments can be avoided, a major benefit for protein immobilization. In addition, the biomolecule immobilization process can be monitored in real time by SPR microscopy. This versatile, multi-channel biomolecule interaction analysis platform is being developed for ADA assessment which will benefit from the ability to implement controls, identify optimal assay conditions, and obtain detailed data about the nature of the biomolecule interactions. Characterization of the integrated system and initial investigations related to ADA assessment will be presented.

2:40pm **BI-MoA3 Transport Properties of Proteins and Quantum Dots in Nanochannels in Multi-Gated Field-Effect-Transistor Configuration**, ***L. Tribby***, University of New Mexico, *F. Van Swol,* Sandia National Laboratories, *C.F. Ivory,* Washington State University, *S.M. Han*, University of New Mexico

The use of nanofluidic architectures as a means of concentrating and separating biomolecules, nanoparticles, and other small species of similar size scale may prove useful in developing new bioseparation and detection technologies. Recognizing this potential, a variety of nanofluidic devices have emerged that utilize enhanced electrokinetic control of fluid and molecular/particle motions at these scales. In our study, we have fabricated an array of slit-like nanochannels (100 nm w x 400 nm d x 15 mm l) in a multi-gated field-effect-transistor configuration, using interferometric lithography and conventional top-down fabrication techniques. Our main objective in developing such a dynamically controllable separation platform is to further increase our ability to rapidly concentrate and separate proteins (or nanoparticles) that have low abundance or require long separation time by conventional methods. In order to produce effective separation strategies, we have first experimentally characterized electrokinetic transport properties of proteins and nanoparticles within our device. Based on this characterization and understanding, we report a technique to form highly concentrated protein bands in our nanochannels. We will also report observable differences in electrokinetic mobility for semiconductor nanocrystals in aqueous solutions whose surface is functionalized with organic ligands to assume different charges. These results and their implications towards nanofluidic separation techniques will be further discussed.

3:00pm **BI-MoA4 High Sensitivity Recessed AlGaN/GaN HFET Protein Sensors**, ***X. Wen***, *H. Kim, P. Casal, S. Lee, W. Lu*, The Ohio State University

We have demonstrated the improvement of sensitivity by biasing the AlGaN/GaN HFET biosensors into the subthreshold regime. To bias the channel into the subthreshold regime, V_g=~-4 V was. The gate voltage causes ions in the physiological buffer solution to move and result in measurement noise. To avoid side effects from a high gate voltage, we adopted the gate recess process to shift the subthreshold gate voltage to zero/near zero volt and retain high sensitivity.

The AlGaN/GaN heterostructure used in this study has a 23 nm thick undoped AlGaN barrier. The recession of AlGaN barrier was conducted with an Oxford Plasmalab 100 system. A two-step recession process was used. The first step uses BCl_3 to etch the AlGaN barrier and the second step uses a combination of $Cl_2/N_2/O_2$ to passivate the etched surface. To achieve zero/near-zero subthreshold gate voltage, we recessed devices with the first step duration to be 50, 55, 60, 65, and 70 sec respectively. We also fabricated diodes with the Schottky area recessed with the same conditions to check the material property changes. The measured C-V characteristics show that the threshold voltage shifts along the positive direction with the increase of etching time. With 70 sec etching time, the threshold voltage of Ni diodes achieve subthreshold regime at Va=0 V. Extracted from C-V curves, the etched depths are 12.0, 11.6, 11.2, 9.7, and 8.8 nm respectively. AFM measurements of both recessed and original AlGaN surfaces show that the surface smoothness is improved after recession processes.

The fabricated AlGaN/GaN HFET biosensors have a recessed gate of 100 um (L) × 2 mm (W). To detect streptavidin, the AlGaN surface was modified by silanization and biotinylation. The measured Id-Vg curves of AlGaN/GaN HFETs with (a) recession time of 65 sec, and without any recession. There are two major differences: 1) the threshold voltage of recessed device shifted to around 0 V; and 2) the off-state current of recessed device is 1-2 orders lower. The threshold voltage promises high sensitivity when the gate is floating or biased at a very low voltage. The decrease of the off-state current indicates that signal-to-noise ratio is improved. The difference between the threshold voltage of diodes and HFETs mainly because of 1) the diode Schottky metal is Ni and the HFET gate is PBS and 2) the HFET is treated with oxygen plasma for surface modifications. The detection of 25 pg/ml (473 fM) SA solution in 1X PBS shows that the drain current is decreased by 22.7% because SA carry negative charges in PBS solution. The sensitivity is increased by one order compared to our previously published results with non-recessed devices.

3:40pm **BI-MoA6 AlGaN/GaN HFETs for DNA Sensing: Charge Layer Distance Dependence**, ***Y. Wang***, *W. Lu*, The Ohio State University

AlGaN/GaN heterojunction features two-dimensional electron gas which is highly sensitive to proximal charges and excellent stability against chemicals. These unique advantages imply the potential of AlGaN/GaN based field effect transistor (FET) in facilitating various biological and chemical studies. We have previously reported the detection of hybridization between probe DNAs and fully complementary target DNAs with AlGaN/GaN HFET biosensors. To further improve the sensitivity, comprehensive understanding of the working principle is necessary. In this work, we focus on a systematical investigation on the effects of the distance between the charged layer and the sensing surface, i.e., the distance from target DNA biomocules to the AlGaN surface. We immobilized a monolayer of single-strand probe DNAs modified with thiol groups on the Au-coated active gate surface of an AlGaN/GaN HFET. Different target ssDNA molecules were designed so that distance between the hybridization sites and the AlGaN surface varied. Experimental results show an explicit relationship between the detection sensitivity and the charge layer distance.

Six types of synthesized 25-mer oligonucletides (Allele) solutions were prepared, including a probe DNA, a fully complementary target DNA, a 21-bp complementary DNA, a 13-bp complementary DNA, a 9-bp complementary DNA and a mismatched DNA. The amount of charges is assumed identical for DNAs with a constant length. The distance of the charge layer can be well controlled by varying the hybridization sites of target DNAs to the probe DNAs. For example, the 21-bp complementary DNA is 4-bp further away from the surface than the fully complementary DNA.

We compared the I_{DS}-V_{DS} characteristics of devices before and after hybridization. A decrease of drain current was noticed, revealing the binding of negatively charged DNAs. More importantly, the decreases in I_{DS}-V_{DS} demonstrated a clear dependence of charge layer distance by an observation of the current change ratio, defined by $\Delta I_{DS} / I_{DS}$, (gate bias: -1 V): 16.1% for fully complementary target DNA (0.1 μ mol), 14.9% for 21-bp complementary DNA, 10.9% for 13-bp complementary DNA, 6.89 % for 9-bp complementary DNA, and 0.75% for mismatch DNA, which was comparably insignificant. The current change decreases with the distance between the target DNA and sensing surface, although the amount of charges for the target DNA is constant. These results demonstrate that the detection sensitivity is significantly dependent on the charge layer distance. Theoretical analysis for varying charge layer distances of target DNAs will also be studied and compared with the experimental results.

4:00pm **BI-MoA7 Effect of Analyte Flow Rate on the Sensitivity of Microcantilever Biosensors**, ***R.P. Desikan***, *C.W. Van Neste, T.G. Thundat*, University of Alberta, Canada

The past decade has witnessed the use of microcantilevers as mechanical transducers of molecular recognition and for the development of miniaturized and sensitive biochip platforms. Microcantilever based biosensors can be based on either mass adsorption or surface stress variation. The potential to operate a microcantilever sensor in liquid medium with extreme sensitivity makes it an ideal choice for the development of biological sensors. Selective detection is obtained by immobilizing receptor molecules on one side of the cantilever. Microcantilever based biological sensors predominantly operate in a liquid environment; this is done in order to retain the functionality of the biomolecules immobilized on the surface. Typically reference cantilevers serve to observe and analyze the effect of non specific interactions and fluid flow rate from specific biomolecular interactions. We have additionally observed that the interaction of analytes on the functionalized surface of the cantilever is influenced by the varying the flow rate of the solution used. The surface stress observed due to the adsorption of analyte molecules on to the receptors on cantilever surface in static condition is much higher compared to a dynamic condition where analyte molecules are allowed to flow across the cantilever surface with the help of a flow control system. Here we address the effect of flow rate on the biomolecular adsorption kinetics of the system and how it affects the sensitivity of Microcantilever based biological sensors.

4:20pm **BI-MoA8 Fabrication of Nanowire FETs for pH Sensing**, ***C. D'Emic***, *S. Zafar, A. Afzali, B. Fletcher, T. Ning, M.A. Guillorn, D.-G. Park*, IBM T.J. Watson Research Center

Sensors for measuring pH are very important for understanding reactions of biological species such as proteins, enzymes and cells. While traditional sensors based upon such techniques as infrared spectroscopy, fluorescence and others have low sensitivity and slow response time, more recent nanowire field effect transistor sensors offer improved sensitivity and response time due to smaller size and increased surface areas. [1, 2]

We have fabricated nanowire FET sensors using conventional CMOS semiconductor processes. The nanowires were patterned by electron beam lithography and reactively ion etched into 30 nm thick silicon on insulator (SOI) substrates. The gate sensing surface is comprised of a hafnium oxide/ silicon dioxide stack covering the nanowire, while the source/drain regions are comprised of boron activated SOI with nickel-platinum silicide contacts. The resulting 16 nm wide nanowire devices show high sensitivity for pH measurements. The FET drain current increases by a factor of eight per unit change in pH, while the subthreshold slope is ~ 77 mV/decade. The sensors operate at a reduced sensing voltage of 0.5 V, making them promising candidates for low power, bio-medical applications.

[1] E. Stern, J. Klemic, et al., Nature, 445 (2007) 519

[2] S. Zafar, C. D'Emic, et al, to be published

4:40pm **BI-MoA9 La^{3+} doped TiO_2 Nano-engineered Platforms for Biosensor**, ***R.R. Pandey***, Centre for Cellular and Molecular Biology, India, *K.K. Saini,* National Physical Laboratory, India, *M. Dhayal*, Centre for Cellular and Molecular Biology, India

The use of nanotechnology tools has opened new opportunities to explore analytical applications of the nano-engineered materials which attracted great attention due to their unique morphology, extraordinary physical and chemical properties towards development of biosensors to facilitate the improvement of the selectivity and sensitivity of the current methods. In this work, the importance of semiconducting La^{3+} doped TiO_2 a metal-oxide-based nanostructure platform is highlighted for biosensors platforms. La^{3+} doped TiO_2 nanostructure having nanometer-scale inner-core cavity which were exposed to the outer surface with different oxidation states having possibilities for redox-activity can make them attractive for sensing uses. Therefore, the use of La^{3+} doped TiO_2 for the development of electrochemical sensors will be discussed. These platforms has been characterized by XRD, XPS, FTIR, SEM, cyclic voltametry to determine structure, surface chemistry and electron transfer characteristics for biosensor applications. Cholesterol oxidase immobilized onto La^{3+} doped TiO_2-based nanostructured surfaces exhibited a pair of well-defined and quasireversible voltammetric peaks in CV measurements. We will also discuss the potential prospect of these surfaces as low cost stable platforms for biomedical diagnosis.

5:00pm **BI-MoA10 Spray Deposition of Functional Antibody Films**, ***J. Figueroa***, *S. Magana, D. Gomez, D.V. Lim, R. Schlaf*, University of South Florida

Antibody films for the use in biosensors and assays are usually deposited via wet-chemical attachment methods. The presented experiments demonstrate that pneumatic spray deposition of antibody thin films from aqueous solution yields films of similar sensitivity and durability without special surface treatments and attachment chemistries. The experiments were performed using a commercially available low flow nebulizer in combination with a syringe pump and a substrate rotation stage to homogenize the coating.

In the experiments E. coli O157:H7 antibody was deposited on cleaned microscopy glass slides without any other pre-treatment. Standard wet-chemically prepared silanized glass slides using the avidin-biotin attachment scheme were also prepared for direct comparison of sensitivity and longevity of the spray based substrates. After incubation with GFP-labeled E. coli O157:H7 cells (ATCC 35150) ranging from 10^4-10^6 CFU/ml the slides were rinsed and AF647-labeled detector antibody was added and incubated. After rinsing and drying the slides were interrogated with a 635 nm laser and visualized using a CCD camera. Slides were also visualized by epifluorescence microscopy to examine antibody patterns and determine E. coli capture efficiencies.

The results of the experiments demonstrate that there is little difference between spray and standard protocol wet-chemically prepared substrates. This indicates that antibody films can be prepared via physisorption without complex attachment chemistries, and that antibodies can directly attach to glass slides, while retaining their functionality.

5:20pm **BI-MoA11 Microfluidic Extraction of Human Chromosomal DNA from Single Cells**, ***J. Topolancik***, *H.C. Tian, C.B. Wallin, D.R. Latulippe, J.J. Benítez, B.R. Cipriany, P.J. Murphy, P.D. Soloway, H.G. Craighead*, Cornell University

Genome-wide analysis of single cells is important in life science research and modern medicine in applications ranging from cancer diagnosis to understanding tissue development. Microfluidic devices have been explored as a promising platform for single cell studies, providing superior handling of minute sample and reagent volumes in engineered microstructures. Isolation of nucleic acids from biological samples is an essential step of every type of genetic analysis. While numerous extraction methods have been explored, it remains rather challenging to isolate and analyze genomic DNA from small cell populations and individual cells. Traditional microfluidic devices utilize solid phase extraction (SPE), a method based on binding of DNA to chemically functionalized solid phase matrices for separation of nucleic acids from cell lysates. The binding affinity is sensitive to factors such as pH, temperature, and buffer composition which must be controlled, often dynamically, to minimize DNA losses. Even when the extraction process is optimized, it is difficult to ensure that all of the DNA fragments are adsorbed on the solid phase matrix and that the whole genome is represented in the purified extracts. An appreciable fraction of genomic DNA is often lost during the purification process when the cell debris is washed away. Additional DNA losses can be caused by incomplete elution. State-of-the-art microfluidic devices for DNA separation from cell lysates exhibit rather modest extraction efficiencies of 60-85%. This is sufficient for genetic analysis of cell populations because multiple copies of every gene are present in the extract, which statistically guarantees complete genome coverage, but such losses are hardly acceptable when single-copy genes in a single cell need to be investigated. This work describes a valveless two-port microfluidic device for highly-efficient isolation and fluorescent analysis of DNA contents of single cells. Long strands of human chromosomal DNA released from the cell by chemical lysis loop around PDMS micropillars and are physically retained while the remaining cellular contents are washed away under hydrodynamic flow. DNA fragmentation is minimized by operating at low flow rates. Hydrodynamic entrapment of DNA in non-functionalized obstacle arrays allows separation of very large genomic DNA from cell debris and components such as proteins and membrane fragments as well as from much smaller mitochondrial DNA and RNA. The purified DNA was subsequently released from the device by enzymatic fragmentation with restriction endonucleases under continuous flow and collected for fragment-size analysis and evaluation of the extraction efficiency. Fluorospectrometric measurements indicate that the microdevice extracts >95% of genomic DNA, which outperforms all alternative microchip-based extraction methods.

Marine Biofouling Focus Topic
Room: 105 - Session MB+BI+PS-MoA

Marine Antifouling Coatings
Moderator: A. Rosenhahn, Karlsruhe Institute of Technology, Germany

2:00pm **MB+BI+PS-MoA1 Advances in Sustainable Technologies for the Prevention of Marine Biofouling**, *R. Deshmukh,* University of Texas at Arlington, *P. Sheth,* University of North Texas Health Science Center, *R.B. Timmons,* University of Texas at Arlington, ***J.A. Schetz***, University of North Texas Health Science Center **INVITED**

The cost associated with preventing the attachment of marine organisms to underwater surfaces (biofouling) is billions of dollars annually impacting numerous sectors including shipping, aquaculture, offshore drilling, and offshore tidal and wind power. Invariably the most effective biofouling control methods are ones that are biocidal in nature whereby a coating is impregnated with a toxin that leaches out over time. However, persistence and bioaccumulation of heavy metal-based and organic biocides, creates environmental and health problems that have resulted in their regulation. Vigorous hull cleaning, aimed at periodically removing biofouling organisms, disperses fine biocide-impregnated coating particles and this significantly increases biocide persistence in sediments where they are ingested by a variety of marine organisms and bioaccumulate.

Coatings that peel off under conditions of high shear, so called foul-release coatings, are another approach but such coatings are easily damaged, not amenable to grooming and ineffective against microfouling. Further, the most effective foul-release coatings are silicon-based and it is becoming increasing apparent that leaching of toxic silicon oils from the coating matrix plays a role in their superior antifouling performance. Other approaches have been to develop durable coatings with little antifouling activity but that can withstand repeated intense grooming. One of our long term objectives is to identify coating materials with surface properties discouraging the settlement of biofouling organisms but also durable enough to withstand cleaning.

Here we report on the discovery of a transparent, conformal, pin-hole free, and plasma-polymerizable polymer coating made from an inexpensive monomer material that drastically reduces (>90%) algae (*Ulva*) sporeling settlement and biomass accumulation without any observable toxicity towards *Ulva* or a non-target brine shrimp species (*Artemia*). Further the unpolymerized monomer has no antifouling effect indicating that potential leaching of monomer from coated surfaces does not account for the observed antifouling activity. Rather the antifouling effect is believed to rely on the presence of a specific chemical moiety because chemical modification of that moiety abolishes antifouling activity. Testing of other marine antifouling species such as microfouling bacteria (*Cellulophaga*) and macrofouling mussels (*Ischadium*) suggests that the antifouling properties of the polymer surface are selective for algae. Potential unique applications for this technology include the coating of algae bioreactor walls or light fixtures allowing for maximal illumination and easy cleaning.

3:00pm **MB+BI+PS-MoA4 Development of a Comparative Protocol for Anti-Fouling Surfaces Based on Polymer Brushes**, ***A. Serrano***, *S. Zürcher, S. Tosatti,* SuSoS AG, Switzerland, *N.D. Spencer*, ETH Zurich, Switzerland

Marine surfaces are known to accumulate fouling material through the starting point of adhesion and settlement of proteins and cells. This effect can be manipulated through the modification and control of the substrate properties via surface functionalization. This approach has led to successful anti-fouling coatings based on biocidal agents containing copper or zinc compounds[1,2]. The environmental toxicity of these latter materials, however, has increased the demand for less adverse coatings. The use of ultra-thin films consisting of polymer brushes has been considered a promising alternative and many studies have been published in this field[3,4]. None, however, has focused on developing a protocol that allows a reliable comparison between the efficiency of different well-known anti-fouling polymers. This is one of the aims of this work and has been achieved by using a common, azide-terminated monolayer to which different non-fouling polymers, such as PEG, PEOXA, PVP and PVA, have been covalently bound. The different materials were compared by characterizing the structure-property relationship of the formed polymeric brushes. Also investigated was the role of the solvent used in the anti-fouling polymer solution as a key element to better control the surface homogeneity. A thorough analysis of the influence of this parameter on the conformation of the final polymer brush was based on ellipsometry, XPS and imaging ToF-SIMS. Finally, the anti-fouling surfaces were subjected to a comparative biological study by exposure to complex proteins solution and *Ulva* zoospores, in order to validate the developed protocol.

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3:40pm **MB+BI+PS-MoA6 Surface Modification of Polymers via Self-Stratification: Decoupling of Bulk and Surface Properties**, ***D.C. Webster***, North Dakota State University **INVITED**

Since materials interact with their surroundings via their surfaces, controlling the surface properties of a material are of critical importance. For materials to be used to mitigate biofouling, having the correct surface properties means the difference between a useful material and one that is unsuitable for the application. In many cases, the material properties of compositions which yield useful surface properties are not suitable for the bulk properties of the material. Thus, being able to decouple the surface and bulk properties is of interest in many areas.

Combining polydimethysiloxane (PDMS) with other polymer systems generally results in materials covered with PDMS due to its low surface energy and incompatibility with other polymers. However, if the PDMS is not chemically bound into the system, it can be easily removed from the surface. Thus, forming a copolymer of the PDMS with the other polymer is required for a durable system. Since polyurethanes are known as tough polymers due to extensive internal hydrogen bonding, combining PDMS with a polyurethane could lead to a material which is tough, but has a low surface energy surface provided by the PDMS. Thus, we have found that incorporating a reactive PDMS into a crosslinked polyurethane system can result in a material which has a low surface energy which is stable when immersed in water. High throughput screening has been used to aid in the identification and optimization of PDMS molecular weight, composition of end groups, and the amount of PDMS in the coating. A unique coating was also identified having discrete domains of PDMS on the surface. Coatings having good fouling-release properties have been prepared and tested in ocean immersion testing.

4:20pm **MB+BI+PS-MoA8 Antifouling Behavior on the Surface of Polyelectrolyte Brushes in Water**, ***M. Kobayashi***, *M. Terada,* Jst, Erato, Japan, *A. Takahara*, IMCE, Kyushu University, Japan

Nature utilizes super-hydrophilic surfaces under wetted state by water to achieve oleophobicity and self-cleaning behavior. For example, fish can maintain a clean body surface by surrounding a thin layer of mucus containing calcium phosphate and protein, which protects oil attachment, marine fouling, and adhesion of marine organisms. In this study, high-density hydrophilic polymer brushes were prepared on Si-wafer by surface-initiated controlled radical polymerization of methacrylate monomers with ionic functional groups.[1] For example, poly(3-sulfopropyl methacrylate potassium salt) (PSPMK) and poly{2-(methacryloyloxy)ethyl phosphorylcholine} (PMPC) brushes with 50 - 100 nm thickness repelled both of air bubble and hexadecane droplet in water.[2] Even when the silicone oil was spread on the polyelectrolyte brush surfaces in air atmosphere, once the oil-sitting brush substrates were immersed in water, the oil quickly rolled up and detached from the brush surfaces due to the low adhesion force between the brush and oil caused by excellent affinity of polyelectrolyte brushes to water. Similar oil detachment behavior was observed on the hydrophilic poly(sodium methacrylic acid) (PMANa) and poly[3-{dimethyl(2'-methacryloyloxyethyl)ammonio} propanesulfonate (PMAPS) brushes,[3] whereas the oil still remained attached on the hydrophobic poly(2-perfluorooctylehtyl acrylate) (PFA-C8) brush even though the PFA-C8 brush shows relatively oleophobic property under air atmosphere. These hydrophilic brush surfaces would contribute to the excellent self-cleaning, antifogging, and antifouling properties without any surfactants

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4:40pm **MB+BI+PS-MoA9 Non-fouling Polymer Chemical Gradients for the Investigation of Marine Bioadhesion**, ***O. Sterner***, ETH Zurich, Switzerland, *S. Zürcher,* SuSoS AG and ETH Zurch, Switzerland, *S. Tosatti,* SuSoS AG and ETH Zurich, Switzerland, *N.D. Spencer*, ETH Zurich, Switzerland

The accumulation of marine organisms on submerged man-made structures (referred to as marine biofouling) has great economical and environmental impact [1]. Numerous strategies to prevent or lower the extent of marine biofouling have been developed, ranging from biocidal coatings to coatings that either prevent adhesion, lower the strength of adhesion or combinations thereof [2-4]. Surface gradients offer a high-throughput approach to investigate the potency of such coatings, and have the additional advantage of reducing the error in experiments by replacing a set of single samples, including positive and negative controls, with a single substrate [5]. In this project, polymeric ultrathin coatings have been prepared using a versatile surface functionalization system based on a self-assembled monolayer of poly(allyl amine) grafted with photo sensitive perfluorophenyl azide functional groups. Gradients have been prepared using a straightforward approach to control the extent of azide to nitrene conversion over the surface, forming a polymer density gradient. Gradients of poly(ethylene glycol), poly(2-ethoxy-2-oxazoline) and poly(vinyl pyrrolidone) have been prepared and investigated for non-fouling action against zoospores from green macrofouling algae *Ulva* and two strains of marine bacteria. The gradients reveal a drastic reduction in bacterial adhesion at low polymer densities for all polymers investigated. Gradients have been characterised with variable angle spectroscopic ellipsometry (VASE) and the properties of the polymer coatings have been evaluated with time-of-flight secondary ion mass spectroscopy (TOF-SIMS) and XPS.

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5:00pm **MB+BI+PS-MoA10 Development of Poly(silyl urethanes) with Tethered Quaternary Ammonium Biocides as Antifouling Marine Coatings**, ***P.N. Coneski***, *N.K. Weise, J.H. Wynne*, Naval Research Laboratory

Due to the significant economic burden posed by high operational and maintenance costs of biofouled ships, the design of environmentally benign, antifouling marine coatings has been a significant interest for many researchers since the ban on traditional coatings was enacted. Current approaches for developing new marine coating materials have primarily focused on the preparation of low surface energy materials, such as modified polydimethylsiloxane (PDMS) and fluorinated polymers. These materials have shown great promise for reducing the adhesion strength of various fouling organisms, thus allowing hydrodynamic forces to release the foulants as the ship moves through water. Unfortunately, no existing material has been shown to eliminate adhesion of all of the numerous different fouling organisms, including zoospores, microalgae and diatomaceous species. As such, the development of marine coatings with multiple mechanisms of fouling prevention may be an important avenue of antifouling materials research. Poly(silyl urethanes) coatings have been developed via the reaction of various quaternary ammonium modified orthosilicates with polyisocyantes. Material properties including glass transition temperature, surface energy, and thermal stability have been investigated as a function of orthosilicate and polyisocyanate composition as well the inclusion or omission of low surface energy soft segments. The low surface energy of these materials should provide excellent fouling release properties, as has been seen with other PDMS-like materials, while the inclusion of bound quaternary ammonium biocides should further reduce the adhesion and propagation of fouling organisms at the material interface. Finally, the hydrolysable silyl ether crosslinking may prove to enhance the antifouling capabilities of these materials by allowing any fouled portions of the coating to slough away over time, generating a new active biocidal interface.

5:20pm **MB+BI+PS-MoA11 A Preliminary Study on Porous Pt-TiO_2/Ti Electrodes with Electrochemically Microbubble-Induced Superhydrophobic Surfaces for Drag Reduction and Antifouling**, ***K.R. Wu***, *C.H. Hung, C.W. Yeh, J.C. Sun, J.K. Wu*, National Kaohsiung Marine University, Taiwan, Republic of China

We investigate a novel device that features a reduction in frictional resistance and antifouling hull surface of seagoing ships which are activated electrochemically by a series of porous Pt-TiO_2/Ti electrodes. This device includes of a series of anodic and cathodic porous Pt-TiO /Ti electrodes insulatedly mounted on the hull surfaces of which are electrically connected to a direct current (DC) power supply. The above-mentioned porous Pt-TiO_2/Ti electrodes are fabricated by two steps; porous TiO_2/Ti plates are firstly prepared on pure titanium plates via a micro-arc oxidation technique and Pt nanoparticles are thereafter deposited on the porous TiO_2/Ti samples using magnetron sputtering. The DC power supply provides an adequate DC bias to the Pt-TiO_2/Ti electrodes where hydrogen and oxygen microbubbles are electrochemically nucleated and formed herein in seawater. As a result, the microbubble-induced superhydrophobic surfaces are created and the reduction in frictional resistance and antifouling hull surface are obtained. Our preliminary tests reveal that no attachment organisms are found on the Pt-TiO_2/Ti electrodes after 15 days of field seawater tests at an applied potential of 1.2 V and an energy consumed rate of about 4 W/m^2. On the other hand, organisms, mainly Crassostrea gigas and barnacles, attached and grew on the Pt-TiO_2/Ti electrode that was not applied a DC potential. That is to say that attachment of organisms can be prevented. Furthermore, the Pt-TiO_2/Ti electrodes yields a gas production rate of 800 cm^3/min m^2 by electrolysis of seawater at an energy consumed rate of about 18 W/m^2. Hence, the microbubble-induced superhydrophobic surfaces can be realized with this gas production rate for drag reduction of the ship hull.

Plasma Science and Technology Division
Room: 202 - Session PS+BI-MoA

Multiphase (Liquid, Solid, Gas) and Biological Related Plasmas

Moderator: A.M. Coclite, Massachusetts Institute of Technology

2:00pm **PS+BI-MoA1 Atmospheric-Pressure Microplasmas for Novel Electrochemical Applications**, ***S.W. Lee***, *R.M. Sankaran*, Case Western Reserve University

Plasmas formed at sub-millimeter spatial scales operate stably and close to non-thermally at atmospheric pressure and are a source of ions, electrons, and other electronically excited states at ambient conditions. Overall, these features make microplasmas suitable for novel electrochemical applications where gas-phase species (e.g electrons) in the plasma can directly interact with ionic aqueous electrolytes to initiate redox reactions.

In this talk, we will present two approaches to microplasma-based electrochemistry that we have developed for nanoparticle synthesis. In one approach, microplasmas are formed at the surface of a liquid electrolyte and operated similar to an electrochemical cell with the plasma as the cathode and a solid metal immersed in the electrolyte as the anode [1]. Metal cations in solution such as Ag+ are electrochemically reduced by the plasma to solid metal, resulting in the formation of metal nanoparticles without any chemical reducing agent. Alternatively, thin films of metal cations dispersed on a polymer are electrochemically reduced by a rastered microplasma [2]. This configuration allows microscale patterns of metal nanoparticles to be produced without the need for lithography. Recently, we have extended our patterning method to the reduction of metallopolymers which are novel molecular structures that can be used as a template for metal ion and metal particle formation [3]. This strategy has enabled patterns of metal nanoparticles to be prepared which are beyond lithographic limits. We will discuss our experimental techniques in detail, as well as the properties of the nanoparticles as assessed by UV-Visible absorbance spectroscopy, X-ray diffraction, and transmission electron microscopy.

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2:20pm **PS+BI-MoA2 Water Containing Non-Equilibrium Atmospheric Pressure Plasmas**, ***P.J. Bruggeman***, Eindhoven University of Technology, the Netherlands **INVITED**

During the last decade water containing non-equilibrium atmospheric pressure plasmas have received a continuously increasing attention in view of their potential in biomedical, environmental, chemical synthesis and material processing applications. This evolution coincides with a strong need for improved diagnostics to enable us to unravel the complex physics and chemistry of water containing plasmas. Both discharges containing a liquid water phase and only water in the vapor phase will be addressed in this contribution.

We will give an overview of the physical and chemical properties of these discharges. The main plasma parameters such as the electron density, gas and electron temperature will be presented and the complications of the interpretation of the diagnostics to obtain these plasma parameters will be discussed. Water containing discharges produce high radical densities such as OH, which is a key radical in several applications. Due to the often high electron density of these discharges recombination reactions are very important not only for radical production but also to explain the optical emission of these discharges. Key differences between liquid water containing discharges and more conventional gas discharges will be discussed.

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3:00pm **PS+BI-MoA4 Mechanism of Au Nanoparticles Formation in Solution Plasma**, ***M.A. Bratescu***, *O. Takai, N. Saito*, Nagoya University, Japan

The Solution Plasma Processing (SPP) has been developed in our laboratory to synthesize nanoparticles and to improve surface properties of carbon nanomaterials by decorating with different nanoparticles or binding functional groups on the surface. The purpose of the present study is to investigate the mechanism of the Au nanoparticles (NP) formation in the SPP and to correlate the SPP properties with the Au NPs characteristics (morphology, size and surface functionalization). The investigation was conducted by changing the solution pH, using the same surfactant and keeping the same processing parameters.

The Au NPs were synthesis in an aqueous solution of 1 mM $HAuCl_4 \cdot 3H_2O$ used as precursor, 1 mM hexadecyltrimethylammonium chloride (CTAC) used as surfactant, and NaOH used to adjust the solution pH (3.2, 6.5 and 11.2). The plasma was produced by using a pulsed high voltage (HV) power supply with the peak voltage and current of 2 kV and 1 A, respectively and the pulse width of about 1 μs. Plasma was characterized by optical emission spectroscopy (OES). The OES gives information about the presence of the relative number densities of the radicals as H, OH, O, and O_2 and from these data the electron temperature and density were evaluated.

On surface, in high vacuum environment, the Au NPs were characterized by Transmission Electron Microscopy (TEM), Secondary Ion Mass Spectrometry Time of Flight (SIMS - ToF) and X-rays Photoelectron Spectroscopy (XPS). In solution, as prepared, the Au NPs were characterized by UV-visible spectroscopy and Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy.

The size of the synthesized Au NPs depends on the initial pH of the solution. In a low pH solution, the particle size was around 15 – 20 nm diameter and in a solution with a higher pH value than 6, the NPs diameter was found to be 2 – 5 nm. The negative SIMS-ToF measurements reveals the presence of the Au^-, $AuCl^-$ and AuN^- ions in the solution with a pH 3 and AuO^- in a solution with pH 11. In a solution before plasma processing, with a low pH value the negative ions Au_2Cl^- and $Au_3Cl_2^-$ were detected, which can suggest an initial agglomeration of Au atoms in solution. The binding functional groups on the Au NPs are confirmed by the XPS analysis.

The correlation among information of the Au NPs morphology, the binding atoms on Au surface, plasma electron temperature and density and the formation of the Au complexes, during the Au NPs synthesis will be presented and discussed.

3:40pm **PS+BI-MoA6 Pulsed Plasma Studies of 2-chloro-p-xylene**, ***I.C. Estrada-Raygoza***, *G. Padron-Wells, P.L.S. Thamban, L.J. Overzet, M.J. Goeckner*, University of Texas at Dallas

Chemical vapor deposited parylene-C is widely used for applications ranging from biomedicine to microelectronics. In our work, we use 2-chloro-p-xylene (2ClpX) as a precursor to deposit plasma polymerized Parylene C. Here we report data aimed at determining the dissociacion mechanisms of the 2ClpX in the plasma. Specifically that data is from: in situ Fourier transform infrared spectroscopy (FTIR); plasma optical emission spectroscopy (OES); and electron beam OES. The main dissociation products are HCl, methane and acetylene. We also observe atomic and molecular hydrogen and chlorine, HCl ion, CH and the characteristic broad aromatic band in the 300 nm region. Both frequency and duty cycle have a strong effect in 2ClpX dissociation. When the pulse period is smaller than the diffusion time, the discharge behaves as continuos wave system, independent of the duty cycle used. When the pulse period is much larger than the diffusion time, the discharge behaves different and very little break up of the monomer is detected. When the pulse period is similar to the diffusion time, then the effect of duty cycle is considerable. This work is supported in part by NSF (Grant CBET- 0922962), Verity Instruments and CONACYT Grant 170201.

4:00pm **PS+BI-MoA7 Deactivation of Lipopolysaccharide and Lipid A by Ar/H_2 Inductively Coupled Plasma**, ***E. Bartis***, University of Maryland, College Park, *T.-Y. Chung, N. Ning, J.-W. Chu, D.B. Graves,* University of California, Berkeley, *J. Seog, G.S. Oehrlein*, University of Maryland, College Park

Low temperature plasma (LTP) treatment of surfaces is a promising path toward sterilization of bacteria [1]. Past works have shown plasma-induced degradation of bacteria [2], but little knowledge exists regarding the plasma-induced chemical modifications in biomolecules that result in inactivation since various plasma species, e.g. ions, reactive radicals, and UV/VUV photons may aid in inactivation. Lipopolysaccharides (LPS) are a main component of the outer membrane of gram-negative bacteria and are difficult to remove from surfaces by conventional methods [3]. LPS is made up of a polysaccharide chain and lipid A and lipid A elicits an immune response in animals [1]. Previous studies have found that adding H2to an Ar plasma leads to a reduction of infrared bands originating from the aliphatic chains of lipid A, namely C-Hx stretching, C-O, and amide bands [4]. This study aims to distinguish the roles of physical sputtering, chemical attack by H-atoms, and plasma-generated VUV. LPS-coated silicon chips were exposed to LTP (Ar, H2, and Ar/H2 mixtures) to explore the effects of plasma composition/ion energy on the etch rates (ER) and chemical and optical properties of LPS. Real-time in-situ ellipsometry was used to monitor ER and changes in the LPS film's optical density during plasma exposure. The real-time data showed that Ar plasmas create a dense film on the surface that decreases in density with H2addition. The films were etched fastest in Ar discharges mixed with ~10% H2and were slowest in pure H2. Since previous work [4] found that adding H2to an Ar discharge enhanced sterilization, these results may indicate that chemical modification rather than rapid erosion may be more important for inactivation. After LTP treatment, samples were characterized by vacuum-transfer to x-ray photoelectron spectroscopy (XPS) to measure the chemical modifications taking place in the LPS layer. With XPS, we measured a decrease in the intensity of the C-C/C-H peak, which indicates that the aliphatic chains in lipid A were removed. The N/C ratio increases approximately equally in all discharges, which suggests that gas chemistry does not have a large impact on amides. Complementary studies with Lipid A will be presented as well as results of a VUV optical filter approach used to probe VUV-induced LPS modifications in real time by in-situ ellipsometry while protecting the material against ion bombardment.

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4:20pm **PS+BI-MoA8 Development of Plasma Treated Mn Induced Nano-arrayed Structures in Sol-gel Derived TiO_2 Matrix for Biosensing Applications**, *R.R. Pandey,* Centre for Cellular and Molecular Biology, India, ***K.K. Saini***, National Physical Laboratory, India, *M. Dhayal*, Centre for Cellular and Molecular Biology, India

We describe Mn doped TiO_2 nanomaterial-based biosensors modified by high pressure RF plasmas for biosensor applications which activates nanostructure matrix and greatly enhanced enzyme loading capacity for development of high sensitivity biosensors. As a test modal, effects of plasma treatment on a novel potentiometric urea biosensor for selective and quantitative recognition of urea by immobilizing urease onto Ti/urease-imprinted Mn induced TiO_2 film has been studied and monitoring the potentiometric response caused by the immobilized urease/urea reaction system was carried out. These platforms has been characterized by XRD, XPS, FTIR, SEM, cyclic voltametry to determine the changes in structure, surface chemistry and electron transfer characteristics of platforms after plasma treatments and have been correlated with improved response of biosensor.

4:40pm **PS+BI-MoA9 Growth Promotion of Bread Yeast using Atmospheric Pressure Dielectric Barrier Discharges**, ***S. Kitazaki***, *K. Koga, M. Shiratani,* Kyushu University, Japan, *N. Hayashi*, Saga University, Japan

Nonthermal atmospheric pressure plasmas have been employed for biomedical processing applications, because they provide high density radicals at a low gas temperature [1]. Recently, nonthermal atmospheric pressure plasmas as well as low pressure plasmas have been employed for growth promotion of plant cells [2,3]. In this study, we have developed a scalable atmospheric dielectric barrier discharge (DBD) device for biomedical processing in a large area and have applied the device to growth promotion of bread yeast. The device consisted of 20 electrodes of a stainless rod of 1 mm in outer diameter and 60 mm in length covered with a ceramic tube of 2 mm in outer diameter. The electrodes were arranged parallel with each other at a distance of 0.2 mm. The dry yeast was set at 1 mm under the electrodes. The discharge voltage and frequency were 10 kV and 10 kHz, respectively. The plasma treatment was carried out in the air. The treatment duration T_{on} was 50, 100 and 150 s. After the treatment, yeast was suspended in 0.5 ml yeast extract peptone dextrose (YPD) medium and agitated with a vortex mixer. 1 µl of sample was mixed with 99 µl YPD medium in a micro well plate and cultivated using a shaking incubator at 30 ^{o}C. To obtain growth curve of yeasts time evolution of 660 nm light absorbance of the samples was measured with a micro plate reader. For the control, the absorbance is almost constant until t = 15 hrs after the beginning of the cultivation, which corresponds to the lag phase, and then it exponentially increases with t, the exponential growth phase. For yeast with the plasma treatment, the absorbance increases from t = 0 hrs. At t = 10 hrs, the maximum absorbance for T_{on} = 150 s is 6.6 times as high as that for the control. From t = 10 to 15 hrs, the gradient of the absorbance becomes gradual. After t = 15 hrs, the absorbance increases exponentially with t. The plasma treatment reduces the lag phase of yeast growth and enhances the growth rate. The growth promotion tends to be enhanced with increasing T_{on} from 50 to 150 s. The growth promotion, therefore, depends on the dose of radicals produced by discharge plasmas.

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5:00pm **PS+BI-MoA10 Plasma Deactivation of Pyrogenic Biomolecules: Vacuum Ultraviolet Photon and Radical Beam Effects on Lipid A**, ***T.-Y. Chung****, *N. Ning, J.-W. Chu, D.B. Graves,* University of California, Berkeley, *E. Bartis, J. Seog, G.S. Oehrlein*, University of Maryland, College Park

Conventional medical instrument sterilization methods are generally ineffective in completely removing harmful biological residues [1]. Biomolecules such as proteins and other pyrogens from bacterial residues are particularly resistant to elevated temperature and are not easily removed by conventional procedures [2, 3]. For example, the presence of lipopolysaccharide (LPS) in host tissue or blood circulation could lead to a generalized sepsis syndrome including fever, hypotension, and respiratory dysfunction and may lead to multiple organ failure and death [4]. Low temperature plasma is a promising technique for sterilization/deactivation of surgical instruments or medical devices, but its effectiveness against such targets is incompletely understood [5]. In this study using a vacuum beam system, we chose lipid A, the major immune-stimulating region of LPS, as a model biomolecule to study. Lipid A consists of a β-1,6-linked D-glucosamine (GlcN) disaccharide carrying two phosphoryl groups. This structure is attached to multiple acyl chains by ester or amide linkage [6]. After vacuum ultraviolet (VUV) photon exposure, loss of CH_2/CH_3, C=O ester, and P=O absorption peaks were observed by ex-situ transmission Fourier transform infrared (FTIR) spectroscopy, but the C=O amide absorption peak was only mildly affected. Monitoring photolysis products from lipid A films by in-situ mass spectrometry, we observed cracking patterns similar to those of alkanes/alkenes with a carbon number ~11-13. This result suggests that VUV photons remove phosphate groups and break ester linkages leading to desorption of acyl chains. Endotoxicity of lipid A is known to be primarily determined by the number and length of acyl chains as well as the phosphorylation state and the disaccharide backbone [6]. The present results therefore indicate that plasma-generated VUV reduces the endotoxicity of lipid A, in support of the hypothesis of Rossi et al. [7]. We report the effects of VUV and radical (H, O) exposures on endotoxicity based upon chemical structural change in Lipid A. Synergism of various beams is compared with plasma exposures and corresponding molecular dynamic (MD) simulations.

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5:20pm **PS+BI-MoA11 Charge Transfer Reactions at the Plasma-Liquid Interface**, ***M. Witzke***, *C. Richmonds, B. Bartling, S.W. Lee, J. Wainright, C.-C. Liu, R.M. Sankaran*, Case Western Reserve University

Electrochemical reactions are normally studied at the interface of a solid metal electrode and an aqueous ionic electrolyte. A smaller number of experiments exist, dating back to more than 100 years ago[1], of plasmas formed at the surface or inside of liquids to initiate electrochemical reactions at the interface of a plasma electrode and a liquid electrolyte. Despite this long history, reactions at the plasma-liquid interface remain poorly understood. Plasmas that are formed at low pressures require liquids with extremely low vapor pressure, limiting previous studies to ionic liquids (i.e. molten salts)[2]. In addition, plasmas are characterized by a complex environment (e.g. ions, electrons, UV, etc.) which has made it difficult to differentiate charge-transfer reactions from other non-faradaic reactions such as radical generation and chemical dissociation.

We have recently developed a novel microplasma source that allows a non-thermal, atmospheric-pressure plasma to be stably formed at the surface of aqueous ionic electrolytes[3-5], facilitating fundamental study of charge-transfer reactions at the plasma-liquid interface. Electron transfer reactions between the plasma and the liquid are studied by using the well-known ferricyanide-ferrocyanide redox couple. The electrochemical reduction of ferricyanide is monitored by UV-vis absorbance spectroscopy and cyclic voltammetry. We find that ferricyanide is indeed reduced by the plasma, confirming that charge transfer reactions can occur at the plasma-liquid interface. The rate of ferricyanide reduction is found to depend on the discharge current, which controls the electron flux delivered to the surface of the solution. By comparing the (discharge) current to the amount of ferricyanide reduced, we obtain a reduction efficiency of ~1%. To address the relatively low efficiency, we have measured the potential at the plasma-liquid interface to determine whether the potential is high enough for water electrolysis and measured hydrogen generation by mass spectrometry. In this talk, we will present our overall methodology and discuss these results in detail.

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***** Coburn & Winters Student Award Finalist

Biofabrication and Novel Devices Focus Topic
Room: 105 - Session BN+NM-TuM

Biofabrication Applications

Moderator: G.F. Payne, University of Maryland, College Park

8:20am **BN+NM-TuM2 Electrically Controlled Biofabrication with Stimuli-Responsive Polysaccharide and Their Visualization in Microfluidic Devices**, ***Y. Cheng***, *X.L. Luo, J. Betz, C.Y. Tsao, H.C. Wu, G.F. Payne, W.E. Bentley, G.W. Rubloff*, University of Maryland, College Park

Stimuli-responsive polysaccharides, such as chitosan and alginate, are useful biomaterials that can be induced to undergo a reversible sol-gel transition to generate biologically-relevant scaffolds. The recent discovery that their gelation can be triggered by imposing an electrical signal opens many avenues for the creation of biologically functional hybrid structures and their localization onto and within microfabricated devices for biofabrication and biosensing applications. Here we report two different mechanisms for creating polysaccharide hydrogels in microfluidics by electrical signal. The cathodic electrodeposition of the cationic chitosan hydrogel was achieved by electrochemically generated OH^- ions at the cathode surface, creating a localized pH gradient at the sol-gel interface. The anodic electrodeposition of calcium alginate hydrogel was achieved by electrical-signal-mediated release of Ca^{2+} ions as a result of electrochemically generated H^+ ions at the anode surface reacting with suspended $CaCO_3$ particles in alginate solution. Localization of the hydrogels in transparent microfluidic devices makes them highly accessible through optical imaging and spectroscopy. The processes of *in situ* gel formation are simple, scalable, spatially controllable, and electroaddressable. Applications in protein immobilization and cell assembly with electroaddressing capability were further demonstrated. With the advantage of spatiotemporal control of gel formation coupled with microfabrication techniques, a variety of novel and useful structures such as multi-layer, multi-address, and even site-programmable arrays of biological components can also be achieved.

8:40am **BN+NM-TuM3 Biofabrication for Interrogating Cell Signaling**, *W.E. Bentley,* ***T. Gordonov***, University of Maryland, College Park **INVITED**

The biological signal transduction process is the means by which external signals are incorporated into information that directly or indirectly alters gene expression and ultimately, phenotype. The hierarchical structure of signal transduction processes is a topic of intense research. Microbial quorum sensing (QS) is responsible for a variety of phenotypes and is rich in diversity and modes of action. As such, quorum sensing represents a "guide" for learning how signals can be translated into altered phenotype.

As microbial communities occupy a confined space over time, concentrations of extracellular signaling molecules accumulate, providing stimulus for unique and varied cellular responses as well as protection from competing microbial communities. Referred to as "quorum sensing" for it's often reported and coincident dependence on high population density, extracellular signaling provides a new basis for control over molecular and cellular processes as well as population behavior, perhaps in a manner more consistent with that of native machinery. Among behaviors guided by QS are the establishment and persistence of bacterial infections.

Our laboratory has uncovered many of the molecular features of the QS autoinducer-2 (AI-2) system using traditional methods that probe bacterial physiology and by exploiting newer princples of biofabrication. That is, we employed electrodeposition methods to assemble complex biological subsystems onto specific sites on microfabricated devices and within microfluidic channels via programmable electrical signals. We have also used genetic engineering techniques to create signal activated fusion tags that covalently link proteins to the device/bio interface. We have designed and synthesized "biological nanofactories" that provide small signal molecule generation at the surface of targeted and captured cells - enabling programmable control of cell function.

Using these methods, we have discovered attributes of the natural switching mechanism that can be exploited for developing next generation antimicrobials. That is, we decomposed elements of the QS "switch" via mutation and a mathematical model of the regulatory elements and coupled this understanding with devices designed to appropriately interrogate these molecular features. Finally, we have developed alkyl analogs of AI-2 that elucidate structural detail and have potential for affecting behavior in natural environments. Correspondingly, these serve as the basis for creating next generation antimicrobials that target the communication between bacteria rather than their survival mechanisms.

9:20am **BN+NM-TuM5 Surface Modified Magnetic Microparticles for Bioreactor Applications**, ***A. Khaing***, *E. Milkani, A. Maziarz, C. Lambert, W. McGimpsey*, Worcester Polytechnic Institute

A magnetically-stabilized, continuous-flow bioreactor was designed and applied for the controlled growth of rat aortic smooth muscle cells (RASMC) in a pre-determined shape in a three-dimensional environment. The cells were immobilized on magnetic agarose beads (MABs) and grown into a tube-shaped tissue. By adjusting the experimental parameters, the size of the MABs were controlled. The surfaces of the MABs were biochemically modified and RASMC cell growth on the modified MABs was tested. Initial RASMC tissue rings with MABs grew in the magnetic field inside the continuous flow of culture medium in the first few days. The RASMC tissue tube was formed in a week, and allowed to mature up to about a month before removing from the bioreactor to characterize it. Histological staining of RASMC tissue tube showed that RASMC were circumferentially aligned perpendicular to the direction of the flow of culture medium. The majority of the cells in the RASMC tissue tube grown out of the MABs stabilized in the magnetic field in the continuous flow were healthy and highly proliferating. The system has applications in the fields of tissue regeneration, pharmaceutical production, stem cell amplification and biofuel production.

9:40am **BN+NM-TuM6 Bacterial Communication in Controlled 3D Microenvironments**, ***X.L. Luo***, *H.C. Wu, C.Y. Tsao, Y. Cheng, J. Betz, G.W. Rubloff, W.E. Bentley*, University of Maryland

Antibiotic resistance is a growing and widely recognized public health issue. Today, more than 70% of bacteria are resistant to at least one of the most commonly used antibiotics. Bacteria evolve with increasing antibiotic resistance due to the selective pressure that administration of conventional antibiotics creates on cell viability, wherein those bacteria that survive antibiotics become dominant. The emergence of "super" bacteria that carry multiple resistant genes calls for the development of novel antimicrobial strategies that place *less* selective pressure on the target bacteria. Rather than killing bacteria with antibiotics, interruption of bacterial communication networks - or quorum sensing (QS) - might delay the population-scale behaviors of target bacteria in gene regulation and buy time for the host immune system to fight back. Microfluidic environments provide a controlled and attractive opportunity to study bacterial QS and to explore these strategies.

Here we report *in vitro* signaling between localized, spatially distinct cell populations in controlled 3D fluidic microenvironments. First, a freestanding chitosan membrane was fabricated by using pH gradients generated at the flow interface of two converging flows. Next, alginate membranes were fabricated by cross-linking alginate sequentially on both sides of the chitosan membrane using diffusion of calcium ions through the semi-permeable chitosan membrane. Finally, cell assembly was achieved by suspending cells in the alginate solution to embed the target cells into the alginate scaffolds, realized as a micro-sandwich structure of cells in alginate on both sides of the chitosan membrane. Signal molecules transmitted *in situ* from one cell population were transported either by diffusion to (1) surrounding cells and (2) nearby segregated cell population, or by convection to (3) cell populations that are relatively far away in a separated microchannel. Induced quorum sensing responses, the production of fluorescence proteins functionally linked to QS genes, were observed for all three configurations. Importantly, these membrane-based 3D scaffolds offer convenient top-down visualization and easy access to both sides of the scaffolds. These approaches provide a versatile and powerful platform to understand and modulate collective and interruptive cellular responses in bacterial quorum sensing.

10:40am **BN+NM-TuM9 "Body-On-A-Chip": Combining Microfabrication, Cell Cultures, and Mathematical Models**, ***M.L. Shuler***, Cornell University **INVITED**

We seek to understand the response of the human body to various pharmaceuticals. Our platform technology is an in vitro system that combines microfabrication and cell cultures and is guided by a computer model of the body. We called this in vitro system a micro cell culture analog (microCCA) or a "Body-on-a-Chip". A microCCA device contains mammalian cells cultured in interconnected micro-chambers to represent key body organs linked through the circulatory system and is a physical representation of a physiologically based pharmacokinetic model. [1, 2] MicroCCAs can reveal toxic effects that result from interactions between

organs as well as provide realistic, inexpensive, accurate, rapid throughput toxicological studies that do not require animals. The advantages of operating on a microscale include the ability to mimic physiological relationships more accurately as the natural length scale is order of 10 to 100 microns.

We have used a microCCA to test potential combination therapies (Tegafur and uracil) for colon cancer. [3] Tegafur is a prodrug for 5-FU and uracil an inhibitor of DPD, an enzyme which deactivates 5-FU. Simple microwell plates cannot probe this system, but the microCCA predicts the types of responses observed experimentally. A "pumpless" system that would be easy to utilize has been demonstrated with Tegafur also.[4] We have coupled these body modules with a micro model of the GI tract to examine the response to oral exposure of drugs, chemicals, or nanoparticles. [5]

Overall, we believe that in vitro, microfabricated devices with cell cultures provide a viable alternative to animal models to predict toxicity and efficacy in response to pharmaceuticals.

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11:20am **BN+NM-TuM11 Simultaneous Bacterial Transformation and Localization within a Microfluidic Device**, ***J. Betz***, *Y. Cheng, C.Y. Tsao, G.F. Payne, W.E. Bentley, G.W. Rubloff*, University of Maryland

Transformation, the process by which a bacterium takes up and incorporates extracellular DNA, is one of the primary enabling technologies in the biotechnology field. This allows a researcher to program bacteria, equipping them with a complement of genes to accomplish a task, such as producing a molecule of interest or acting as a sensor. We describe the simultaneous transformation and localization of *Escherichia coli* bacteria in response to an electric signal within a microfluidic device. We demonstrate that these transformed bacteria can act as fluorescent sensors of isopropyl β-D-1-thiogalactopyranoside (IPTG), a chemical stimulus, or low dissolved oxygen levels, an environmental stimulus.

This method focuses on bacterial transformation with the added benefit of simultaneous entrapment within an alginate hydrogel at a desired electrode address. This offers the ability to create microfluidic cell-based sensors in a single, simple step. To transform and deposit bacteria, the device was filled with a mixture of electrocompetent cells, 200ng plasmid, 0.5% alginate, and 0.125% $CaCO_3$ and subjected to a 30V/cm DC electric field for 3 minutes on ice. The cells were allowed to recover at 37ºC for an hour, cultured for 16 hours, and induced with a chemical signal, IPTG, for 4 hours. This resulted in increased expression of DsRed, a red fluorescent protein.

Dissolved oxygen is an important parameter for many cell culture experiments. To create a dissolved oxygen sensor, *E. coli* were transformed with a plasmid that causes production of green fluorescent protein (GFP) in response to decreased dissolved oxygen concentration in the surrounding medium. Following the above transformation and culturing method, the cells were induced with media that had been deoxygenated in a vacuum chamber, resulting in an increase in GFP expression.

This method is versatile in terms of creating microfluidic cell-based sensors. We envision many exciting applications of this work, including the development of dynamically reconfigurable microfluidic biosensors and high-throughput screening methods for plasmid libraries generated by protein engineering and directed evolution experiments.

Biomaterial Interfaces Division
Room: 105 - Session BI-TuA

Protein-Membrane Interactions

Moderator: L.J. Gamble, University of Washington

4:00pm **BI-TuA7 Membrane Binding, Structure and Regulation of the PTEN Phosphatase**, ***M. Lösche***, Carnegie Mellon University and National Institute of Standards and Technology **INVITED**

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is an important regulatory protein and tumor suppressor that performs its phosphatase activity as an interfacial enzyme at the plasma membrane-cytoplasm boundary. Acting as an antagonist to phosphoinositide-3-kinase (PI3K) in cell signaling, it is deleted in many human cancers. Despite its importance in regulating the levels of the phosphatidylinositoltriphosphate $PI(3,4,5)P_3$, there is little understanding of how PTEN binds to membranes, is activated and then acts as a phosphatase. The interaction of the protein with membranes is highly dynamic and is at least partially controlled by the in-plane fluidity of the bilayer. PTEN function requires multiple, lipid-specific interactions with the target membrane. These interactions regulate enzyme activity as well as lateral and subcellular distribution of the enzyme. From studies of the membrane association of PTEN under well-defined conditions in model systems, we report recent insights in the structural and functional basis for PTEN membrane binding and regulation.

4:40pm **BI-TuA9 Biomimetic Lipid Membrane Systems Applied to HIV-1 Neutralization**, ***G. Hardy***, *M. Alam, S. Zauscher*, Duke University

Evidence suggests that lipid membrane interactions with rare, broadly neutralizing antibodies (NAbs), 2F5 and 4E10, play a critical role in HIV-1 neutralization. The **objective** of this research is to understand how lipid membrane properties, such as chemical head groups, lipid domain organization, and lipid diffusivity contribute to 2F5/4E10 membrane interactions and antigen localization at the membrane interface, with the ultimate vision of guiding immunogen designs. Recent immunization studies have shown that induction of antibodies that avidly bind the gp41-MPER antigen is not sufficient for neutralization. Rather, it is required that antigen designs induce polyreactive antibodies that recognize MPER antigens as well as the viral lipid membrane. However, the mechanistic details of how membrane properties influence NAb-lipid and NAb-antigen interactions remain unknown. **Methods:** To understand how membrane properties contribute to 2F5/4E10 membrane interactions, we have engineered biomimetic supported lipid bilayers (SLBs) and have developed a surface plasmon resonance (SPR) spectroscopy based assay that monitors antibody binding to thiol monolayers, which mimic salient surface chemical properties of lipid membranes.

Our **results** showed that 2F5 and 4E10 bound preferentially on charged and hydrophobic thiol surfaces. This supports the theory that NAbs interact with lipid head groups before embedding into hydrophobic tail regions. We have also engineered supported lipid bilayers (SLBs) whose compositions mimic both the host cell membrane and the HIV-1 envelope. These SLBs have planar surfaces that facilitate quantitative surface-characterization techniques such as high-resolution scanning-probe imaging, detection of fluorescence recovery after photobleaching, and neutron reflection measurements. Using these characterization techniques we have begun to i) visualize domains of lateral membrane organization; ii) determine SLB domain diffusivity; iii) determine differences in adhesion force (surface energy) of domains; and iv) correlate these membrane properties with NAb-membrane binding and NAb/antigen localization.

Our research is **significant** in that it provides a biologically relevant system to screen interactions of lipid-reactive antibodies with a broad range of diagnostic tools. Because current 2F5/4E10 immunogens have not yet elicited antibodies with the required membrane reactivity it is important to reveal the role of lipids underlying antibody-antigen binding. This information will elucidate how membrane properties can enhance antigen recognition and thus enable the design of next generation HIV-1 immunogens.

5:00pm **BI-TuA10 Interactions between the Norovirus and Glycosphingolipids Studied with Cell Membrane Mimics**, ***M. Bally***, Chalmers University of Technology, Sweden, *G. Larson,* University of Gothenburg, Sweden, *F. Höök*, Chalmers University of Technology, Sweden

The determining initial step of viral infection is mediated by highly specific recognition events between the viral shell and ligands on the host cell surface. Detailed understanding of virus-membrane interactions is therefore of central importance to the development of new antiviral therapies, new vaccines and high-performance diagnostics platforms. In this context, assays based on cell-membrane mimics have a considerable potential, as they offer the possibility to study interactions between controlled ligand mixtures with surface-sensitive techniques, while presenting the ligand in a more native configuration. Potentially relevant characteristics such as membrane fluidity, ligand mobility and their ability to organize into microdomains can be preserved [1].

In this work we investigate the interactions between norovirus capsids and phospholipid bilayers containing glycosphingolipids (GSL). The norovirus is well known as the major causative agent of acute viral gastroenteritis, but its human target cells and the precise mechanism for viral entry are still poorly understood.

In a first example, we identify galctosylceramide - a major glycosphingolipid in the small intestine- as a ligand for Norovirus-like particles (VLP) from the Dijon strain. Quartz Crystal Microbalance and Atomic Force Microscopy studies on GalCer-containing supported lipid bilayers reveal that a clustered arrangement of the glycosphingolipids plays a crucial role in promoting a firm attachment of the pathogen to the lipidic membrane, most likely via the establishment of multiple contacts between the particle and the membrane [2].

We further investigated the interaction between individual fluorescent GSL liposomes and surface immobilized VLPs with single virus particle sensitivity. Besides representing the ultimate sensitivity for diagnostics purposes, our method makes it possible to study weak interactions. Kinetics analysis of vesicle residence times over large time scales reveals a highly heterogeneous behavior and yields information on multivalency, on the presence of domains and the role of cell-membrane curvature.

As exemplified here, simplified membrane models have a unique potential in providing fundamental understanding on the contribution of individual components to complex biological processes [3].

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5:20pm **BI-TuA11 Binding of C-reactive Protein to Lipoprotein Nanoparticle Mimics: A Gel Electrophoresis Study**, ***M.S. Wang***, *S.M. Reed*, University of Colorado Denver

C-reactive protein (CRP) is an acute phase serum protein involved in inflammation that recognizes pathogenic agents, and activates complement. Because CRP levels can increase by over 1000-fold from basal levels within 72 hr in response to inflammation, it has been used as a biomarker to predict the risk for cardiovascular disease (CVD). In addition, CRP has been shown to bind oxidized low density lipoprotein (oxLDL) through the phosphatidylcholine (PC) headgroup that is exposed on the surface of oxLDL and has been found to co-localize with atherosclerotic lesions. It is suggested that CRP was deposited in these lesions when engulfed by macrophage while it is still bound to oxLDL. Because LDL and the other lipoprotein particles (LPPs) have diameters (d) in the nanometer range, they can be considered as biological nanoparticles. In fact, LPPs are classified into four major categories according to their size and density: high density lipoprotein (d=8-13nm), LDL (d=20-30nm), intermediate density lipoprotein (d=30-40nm), and very low density lipoprotein (d>40nm). Moreover, the physiological functions of LPPs are greatly influenced by the size. For example, the presence of sdLDLs (d<25.5nm) are associated with increased risk of coronary artery disease (CAD) and diabetes; while increased levels of HDL are considered to be atheroprotective. Therefore, LPP profiling has emerged as a tool to more accurately assess the development of metabolic risk factors such as CAD. While the PC moieties on oxLDL are likely ligands for CRP recognition, it is still unclear why CRP binds only oxLDL although PC is expressed on native LDL and on all cell membrane. In this work, we correlated the effects of particle size (i.e. membrane curvature) to CRP binding using LPP mimics. To this end, we engineered LPP mimics using lipid-coated gold nanoparticles (PC-AuNPs) to explore the influence of LPP size on CRP binding. Binding analysis of CRP to the mimics was performed using gel electrophoresis (GE). The migration of CRP and PC-AuNP was directly visualized after electrophoresis, and the presence of CRP was confirmed using Western blots. The overlapping bands from the gel and Western blot confirmed that CRP bound to the PC-AuNPs and co-migrated during GE. Together, we demonstrated that 1) PC-AuNPs are size-separable, 2) the lipid layer around

the PC-AuNP remained intact during GE, and 3) CRP binds to a relevant LDL-sized PC-AuNP mimic. This is, to our knowledge the first use of GE to separate lipid-coated nanoparticle and to evaluate non-covalent binding of protein-nanoparticle interactions.

Biofabrication and Novel Devices Focus Topic
Room: 105 - Session BN-TuA

Biofabrication Methods and Devices
Moderator: L. Gamble, University of Washington

2:00pm **BN-TuA1 Microengineered Hydrogels for Stem Cell Bioengineering and Tissue Regeneration**, ***A. Khademhosseini***, Brigham and Women's Hospital, Harvard Medical School, MIT, and Harvard University **INVITED**

Micro- and nanoscale technologies are emerging as powerful tools for controlling the interaction between cells and their surroundings for biological studies, tissue engineering, and cell-based screening. In addition, hydrogel biomaterials have been increasingly used in various tissue engineering applications since they provide cells with a hydrated 3D microenvironment that mimics the native extracellular matrix. In our lab we have developed various approaches to merge microscale techniques with hydrogel biomaterials for directing stem cell differentiation and generating complex 3D tissues. In this talk, I will outline our work in controlling the cell-microenvironment interactions by using patterned hydrogels to direct the differentiation of stem cells. In addition, I will describe the fabrication and the use of microscale hydrogels for tissue engineering by using a 'bottom-up' and a 'top-down' approach. Top-down approaches for fabricating complex engineered tissues involve the use of miniaturization techniques to control cell-cell interactions or to recreate biomimetic microvascular networks within mesoscale hydrogels. Our group has also pioneered bottom-up approaches to generate tissues by the assembly of shape-controlled cell-laden microgels (i.e. tissue building blocks), that resemble functional tissue units. In this approach, microgels were fabricated and seeded with different cell types and induced to self assemble to generate 3D tissue structures with controlled microarchitecture and cell-cell interactions.

2:40pm **BN-TuA3 Nanoscale Architectures for Probing Cell Mechanics**, ***S. Wind***, *M. Schvartzman, M. Palma, M. Biggs, T. Fazio, R. Piqueras Jover, M. Sheetz*, Columbia University

The physical properties of a cell's environment are important factors in determining cell behavior and ultimately, phenotype. Two key factors that have been associated with major changes in cell morphology and behavior are (1) spatial organization of extracellular matrix (ECM) molecules and (2) rigidity. In order to understanding how cells sense these factors at the nanoscale and how these factors affect cell function, we have developed new nanofabricated surfaces in which these physical characteristics of the ECM are simulated.

The first type of surface combines nanoimprint lithography with selective biofunctionalization to precisely control the placement and geometric arrangement of integrin binding sites. The binding sites consist of sub-10 nm metallic nanodots functionalized with ECM binding ligands, designed so that each site can accommodate only a single integrin molecule. Cell spreading and motility assays were performed using 3T3 fibroblasts on arrays in which binding site spacing, density and number were independently varied. Cell spreading efficiency was markedly enhanced for clusters comprising at least 4 liganded sites spaced $\leq$ 60 nm apart, with little or no dependence on global density. This points to the existence of a minimal matrix adhesion unit defined in space and stoichiometry.

A second type of surface consists of elastomeric substrates with locally variable rigidity. We have found that exposure of poly(dimethylsiloxane) (PDMS) to an electron beam alters the rigidity of the elastomer, with the modulus of the exposed regions increasing with the applied electron dose. In addition to planar surfaces, pillared substrates can be patterned with no measurable change to the pillar dimensions. Immortalized mesenchymal stem cells plated on soft PDMS surfaces patterned in this manner displayed a distinct preference for the more rigid, exposed regions, forming focal adhesion nearly exclusively there. Furthermore, focal adhesion formation diminished significantly as the size of the exposed features was reduced below 1 μm, indicating that there is a length scale for cellular rigidity sensing, with the critical length in the range of a few hundred nanometers.

By adapting the tools of nanomanufacturing to cellular systems, we are able to define important parameters that can control aspects of cell function and behavior and will help identify conditions under which these functions may be altered. Potential applications range from therapeutic treatments that block metastasis to the development of new adoptive immunotherapies, as well as the development of new guidelines for the design of tissue scaffolds that can optimize healing without scarring.

3:00pm **BN-TuA4 Production of Functionalized 3D Micro Environment for Cell Culture**, ***J. Nowak***, *D. Mehn, P. Colpo, M. Zurn, T. Martin, F.J. Rossi*, European Commission, JRC Institute for Health and Consumer Protection, Italy

One of the main challenges for the robust *in-vitro* studies is to obtain adaptable 3D culture systems that may mimic the tissue environment. Unfortunately the universal condition used in 2D cell culture techniques may hinder the full functionality of cells and generate misleading results.

Fabrication of firm and flexible micro-structures from organic polymers offers benefits for making smart 3D environments capable of driving cell behavior and surpassing the limitations of the 2D systems. These 3D bio-scaffolds can be employed to study various aspects of cell biology. Furthermore upon functionalization with the extra-cellular matrix proteins or signaling molecules they can be used as platforms for governing stem cell differentiation into the specialized cell types.

Here we present the straightforward approach to generate 3D bio-scaffolds that can facilitate cell growth under controlled geometrical and chemical conditions.

The technique involves UV cross-linking of the polymeric precursors to create the micro-well structures. The geometrical features of the structures are obtained by introducing a physical mask in contact with a liquid precursor, therefore restricting the region of the polymerization. We used PDMS mold as a physical mask to direct the polymerization of the PEG-DA and epoxy based polymers. However the technique can be used with various UV-sensitive polymeric materials.

The chemical and geometrical properties of the structures were characterized by XPS and microscopic techniques.

The features of the scaffolds lead to the development of a geometrically defined neuronal network when applied as platforms in a primary-neuron culture. Cell morphology and expression of the neuronal markers were characterized by fluorescent microscopy.

Applied Surface Science Division
Room: 102 - Session AS+BI+NS-WeM

Advances in Scanning Probe Microscopy
Moderator: S.A. Allen, The University of Nottingham, UK

8:00am **AS+BI+NS-WeM1 High-Speed Atomic Force Microscopy for Filming Biomolecular Processes**, ***T. Ando***, Kanazawa University, Japan **INVITED**

Vital phenomena are engendered through the dynamic activity of biological molecules. Therefore, observing the dynamic behavior of biological molecules in action at high spatiotemporal resolution is essential for elucidating the mechanism underlying the biological phenomena. The dynamic biomolecular processes are now widely studied using single-molecule fluorescence microscopy. However, the fluorescently labeled biological molecules themselves are invisible in the observations even using super-resolution fluorescence microscopy. The structure of biological molecules has been studied using x-ray crystallography, NMR, electron microscopy, and atomic force microscopy (AFM) but the obtained structures are essentially static. Thus, the simultaneous assessment of structure and dynamics is infeasible. To overcome this long-standing problem and make it possible to simultaneously record the structure and dynamics of biological molecules, we have been developing high-speed AFM for more than 15 years and at last it is now coming of age. Various AFM devices and control techniques were optimized or invented for high-speed scanning and low-invasive imaging. As a result, the imaging rate now reaches 10-30 frames/s for the scan range 250 ×250 nm^2, 100 scan lines, and the spatial frequency of a sample surface corrugation 0.1/nm [Prog. Surf. Sci. **83**, 337-437 (2008)]. Remarkably, even delicate protein-protein interactions are not disturbed by the tip-sample contact. With this capacity of high-speed AFM, some biological processes are successfully captured on video, such as walking myosin V molecules along actin filaments [Nature **468**, 72-76 (2010)], photo-activated structural changes in bacteriorhodpsin [Nat. Nanotechnol. **5**, 208-212 (2010)], and cooperative GroEL-GroES interactions. The high-resolution movies not only provide corroborative 'visual evidence' for previously speculated or demonstrated molecular behaviors but also reveal more detailed behaviors of the molecules, leading to a comprehensive understanding of how they operate. Thus, the high-speed AFM imaging of functioning biological molecules has the potential to transform the fields of structural biology and single-molecule biology.

8:40am **AS+BI+NS-WeM3 Integrated Imaging: Probing Molecular Interactions by Correlated Atomic Force Microscopy Approaches**, ***C. Yip***, University of Toronto, Canada **INVITED**

The development of powerful single molecule functional imaging tools has been critical to our understanding of molecular dynamics and structure-function relationships in (bio)molecular systems. Our lab's focus on the design, implementation, and application of coupled imaging and spectroscopy is providing intriguing insights into the mechanisms of membrane disruption, receptor oligomerization, and protein-membrane interactions. We have devised several correlative approaches based on the integration of in situ atomic force microscopy with fluorescence and vibrational spectroscopies for extracting the orientation, conformation, and association dynamics of membrane-associated proteins in model membranes and in live cells. Some of the key challenges and opportunities afforded these new tools will be discussed.

9:20am **AS+BI+NS-WeM5 Visible Light Emission from Fluorescent Proteins on Silver Substrate Induced by Tunneling Electrons**, ***T. Yamada***, RIKEN, Japan, *T. Iwaya, S. Matsunaga, M. Kawai*, The University of Tokyo, Japan

We detected the characteristic visible light emission from fluorescent protein molecules deposited on metallic silver (Ag) upon injection of tunneling electrons generated by a standard scanning tunneling microscope (STM) in ambient condition. A series of fluorescent proteins originating from jellyfish or coral, nowadays engineered to generate various colors of fluorescence by gene technology, is characterized with a β-barrel structure insulating the chromophore electronically from the surrounding. We purchased green, yellow, red and infrared fluorescent proteins (GFP, YFP, RFP, HcRed, molecular diameter ≈ 5 nm), deposited on a bare Ag surface, and used a Ag tip set on a STM setup to obtain images and to generate fluorescence. Light from the gap was collected by an optical fiber and introduced to a grating spectrometer with a liquid N_2-cooled CCD detector. On bare Ag surfaces, visible light was detected with the STM bias voltage within ±1.8 V in a modestly moisturized N_2 atmosphere. The spectra were unstable in general, indicating light emission upon excitation of local plasmon [1], which depends on the changeable geometry of Ag tip. The wavelength onset of emitted light was equivalent to the STM bias voltage within ±3.0 V, obeying the law of quantum energy conservation. The fluorescent proteins were dissolved in pure water, drop-cast on the Ag substrate and air-dried to form multilayers. STM images mostly showed flat terraces with steps composed of the protein molecules. Within a 200nm x 200nm scanning area, the light emission spectra apparently involved the characteristic fluorescence peaks of proteins (GFP = 540 nm (2.30 eV), YFP = 550 nm (2.25 eV), RFP = 650 nm (1.91 eV), HcRed = 660 nm (1.88 eV)) over a background of weakened Ag plasmon spectrum. The same experiment with Au tips and Au(111) substrates was with almost no detection for the characteristic fluorescence of all the proteins. For clean Au(111), although visible light was detected, the above-mentioned plasmon energy conservation stood for the bias voltage only within ±1.9 V. The maximum energy of local plasmon on Au(111) is too small to excite the florescent proteins electronically. The characteristic fluorescence from proteins is considered aided by the plasmon excitation of the Ag substrate. The protein β-barrel structure reserves the lifetime of excited state towards light emission, insulating electronically from the metallic substrate against the radiationless de-excitation process of the present surface-adsorbate system.

References:

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9:40am **AS+BI+NS-WeM6 Characterization of Peptide Nanotubes by Atomic Force Microscopy**, ***J.L. Remmert***, *M.C. Vasudev,* Air Force Research Laboratory, *L. Eliad, E. Gazit,* Tel Aviv University, Israel, *T.J. Bunning, R.R. Naik, A.A. Voevodin*, Air Force Research Laboratory

This work investigates the properties of aromatic dipeptides, which are of interest due to their ability to self-assemble into nanotubes and nanowires. Peptide nanotubes have been used to template inorganic materials[1] and construct nanochannels in microfluidic devices[2]. The mechanical, thermal, and electronic transport properties of these nano-structures are desired to evaluate their potential use for biomolecular electronics[3] and other applications. Atomic Force Microscopy (AFM) offers multiple modes to interrogate the response of discrete nanotubes. For instance, AFM with dry sample heating has established the thermal stability of peptide nanotubes up to 100 °C[4] with a spring constant of 160 N/m at room temperature[5]. A separate study targeting a single nanowire bridging two electrodes revealed semiconductor characteristics under repeated bias cycling[6]. We have similarly sampled detached nanotubes among peptide bundles and vertically aligned 3D arrays. Peptide nanotubes were synthesized by either Plasma Enhanced Chemical Vapor Deposition (PECVD) or solvent phase growth in 1, 1, 1, 3, 3, 3 Hexafluoroisopropanol (HFP), using approaches similar to that described by Reches et al[1]. The nanotubes were observed by SEM to vary between 85-100 nm in diameter and up to 50 μm in length. Sample density was controlled by suspension and dilution in various solvents, including HFP and water, prior to deposition on a variety of substrates. AFM studies have revealed details of the tubular outer shell with tapping and electrostatic force modes (EFM), while also probing the mechanical integrity and thermal response to localized tip-side heating.

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[2]N. Sopher, Z. Abrams, M. Reches, E. Gazit, Y. Hanein, "Integrating peptide nanotubes in micro-fabrication processes", J Micromech Microeng 17 2360 (2007)

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[4]V. Sedman, L. Adler-Abramovich, S. Allen, E. Gazit, S. Tendler, "Direct Observation of the Release of Phenylalanine from Diphenylalanine Nanotubes", J Am Chem Soc 128 6903 (2006)

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10:40am **AS+BI+NS-WeM9 Determination of Molecular Polarization at Protein-Electrode Interfaces with Combined Optical, Transport, and Dielectric Scanning Probe Microscopy**, ***X. Chen***, *K. Kathan-Galipeau, B.M. Discher, D.A. Bonnell*, University of Pennsylvania

Bio-molecule integrated electronic devices are of great interest recently. For such systems to be designed and fabricated, the optoelectronic properties of protein molecules in ambient environment must be understood at a fundamental level. Here we demonstrate a new scanning probe based technique: torsional resonance nanoimpedence microscopy (TR-NIM), which simultaneously probes transport and dielectric properties in conjunction with optical excitation. To make a controlled interface, we start by designing a peptide molecule with ability to control protein/electrode interface interactions, as well as incorporation of several different optically active cofactors, and we successfully patterned peptides on HOPG substrates. Using TR-NIM electronic transport and the effect of optical absorption on dielectric polarizability in oriented peptide single or multiple molecular layers is determined. This approach enables quantitative comparisons of the change in polarization volume between the ground state and excited state in both single and multiple molecular layers.

11:00am **AS+BI+NS-WeM10 Scanning Local Capacitance Measurements with High Spatial and Dielectric Resolution**, ***M.J. Brukman***, *S. Nanayakkara, D.A. Bonnell*, University of Pennsylvania

Spatial variation of dielectric properties often dictates the behavior of devices ranging

from field effect transistors to memory devices to organic electronics, yet dielectric

properties are rarely characterized locally. We present methods of analyzing 2nd

harmonic-based local capacitance measurements achieved through non-contact atomic

force microscopy. Unlike contact-based methods, this technique preserves tip

shape and allows the same probe to realize high-resolution topographic imaging and

scanning surface potential imaging. We present an improved analysis of the electrical

fields between tip and sample, yielding high sensitivity to the capacitance-induced

frequency shift.

The techniques are applied to thin-film strontium titanate and mixed-phase self-

assembled monolayers to illustrate application to high dielectric constant hard materials

and lower dielectric constant organic films. Conversion from frequency shift signal to

dielectric constant κ is demonstrated on both samples, with sub-5 nm spatial resolution

and dielectric constant resolution between 0.25 and 1.

11:20am **AS+BI+NS-WeM11 Parallel Momentum Conservation of Hot Electrons across a Metal Semiconductor Interface**, ***J.J. Garramone***, *J. Abel, R. Balsano, V.P. LaBella*, College of Nanoscale Science and Engineering, the University at Albany-SUNY

Parallel momentum of electrons is a conserved quantity as the electron traverses a barrier between two materials which lead to refraction like effects in the electrons trajectories. Ballistic electron emission microscopy (BEEM) is a scanning tunneling microscopy (STM) based technique that injects hot electrons (E>EF) into a metal-semiconductor Schottky diode[1]. A small fraction of these electrons will traverse the metal with little to no scattering and make it into the semiconductor and counted as BEEM current. This makes it an ideal technique to study parallel momentum conservation. However, direct observation of this effect has been rather elusive. To observe this effect the dependence of the attenuation length with hot electron energy of Ag on both the Si(001) and Si(111) substrates has been measured.

Samples consisted of nanometer thick Ag films that were deposited on HF cleaned Si(001) and Si(111) wafers and capped with 10 nm Au to prevent oxidation of the films. Attenuation lengths were extracted by measuring the BEEM current as a function of the metal overlayer thickness. The dependence of the attenuation length with tip bias (electron energy) displayed a sharp increase as the energy approached the Schottky barrier height for the Si(001) substrates and a slight decrease for the Si(111) substrates. This contrast is a direct result of parallel momentum conservation and the lack of zero parallel momentum states at the Si(111) interface when compared to the Si(001) interface. Additional insight into the relative contribution of both elastic and inelastic scattering can be obtained by fitting the data to a Fermi liquid based model.

[1] L. D. Bell, et al., Phys. Rev. Lett. **61** 2368 (1988).

11:40am **AS+BI+NS-WeM12 High Resolution Scanning Probe Imaging of 2D-Supramolecular Networks on Au(111), Graphite and Molybdenite**, ***V.V. Korolkov***, *S. Allen, C.J. Roberts, S.J.B. Tendler*, The University of Nottingham, UK

Chemical decoration of surfaces with various molecules and supramolecular structures has been a major strategy for introducing new properties to both organic and inorganic materials. Amongst these properties are wettability, biocompatibility, sensing properties, catalytic activity, optical properties and adhesion. Most of methods for surface modification include molecules binding to the surface via stable chemical bond. Recently methods have been developed to modify atomically flat surfaces with periodical porous molecular structures, termed 2D-supramolecular networks. The networks are commonly composed of two types of molecules serving different functions e.g. joints and ribbons. Such 2D-structures bring forward a unique surface property - a spatially controlled adsorption with almost single molecule precision.

Most networks reported in the literature have been studied using UHV STM on metal substrates and, to a lesser extent, on HOPG and non-conductive substrates. Here we present a study, utilizing both ambient STM and AFM, of 3,4,9,10-perylenetetracarboxylic diimide (PTCDI) - melamine networks deposited on Au(111), HOPG and MoS_2 substrates. AFM imaging was performed using PeakForce Tapping AFM (Bruker Inc.) and Torsion Resonance (TR)-AFM. Both STM and AFM were able to resolve a clear periodical network structure for all substrates after exposure to a solution of PTCDI and melamine molecules in dimethylformamide at 373K. AFM images show that the network forms a monolayer on both HOPG and molybdenite substrates, and also that most of the HOPG surface is covered with network structure, with some minor defects. In contrast the Au(111) surface was mostly covered with network multilayers as suggested both by TR-AFM and STM. AFM also revealed that the network structure on HOPG and molybdenite remains intact for several hours in the ambient and can be stored in N_2-ambient for up to ~24h.

Biomaterial Interfaces Division
Room: 108 - Session BI-WeM

Cells at Interfaces
Moderator: M.R. Alexander, University of Nottingham, UK

8:00am **BI-WeM1 Real Time Analysis of Polymer Film Integrity Upon Exposure to Bacteria and Aqueous Medium**, ***D.E. Barlow***, *J.C. Biffinger,* Naval Research Laboratory, *E.R. Petersen,* Nova Research, Inc., *J.N. Russell, P.E. Pehrsson,* Naval Research Laboratory, *W.J. Goodson*, Air Force Resarch Laboratory

Polymer coatings are of great importance for protecting and imparting specific properties at the surfaces of man-made structures, but can be affected in many ways by the natural environments they must withstand. We have studied the effects of aqueous medium exposure and biofilm formation on antistatic polyurethane coatings in real time using in situ ATR-FTIR. The results show that the coatings are susceptible to water permeation and swelling, and deuterium exchange was also shown to occur within the films upon D_2O exposure. When exposed to *Pseudomonas fluorescens* in M9 minimal medium, the coating interface became compromised as the pyruvate carbon source was depleted. Reasons for these changes will be discussed, including the role of water permeation and the potential for the bacteria to use the coating as a carbon source. While ATR-FTIR has been used in the past to study biofilm growth, these results also demonstrate the effectiveness of the method for assessing substrate impact, an often overlooked factor.

8:20am **BI-WeM2 Early Stages of Bacterial Biofilm Formation – A Numerical Study of Bioadhesion on Biomaterials**, ***D. Siegismund***, *A. Schroeter, S. Schuster, M. Rettenmayr*, Friedrich Schiller University Jena, Germany

Biomaterials for implant purposes are increasingly applied in modern medicine e.g. to recover human body functions or for tissue substitution in general. Infections of these implants, called Biomaterial-centered infections

(BCI), are among the fundamental challenges in biomaterials science. They are primarily initiated by adhesion of bacteria on the biomaterial's surface. The subsequent formation of a bacterial biofilm requires a total implant replacement in the majority of cases.

The adhesion of bacteria is thus the first crucial step for biofilm formation that is only incompletely understood. Interactions of bacteria with the surface are controlled by surface properties such as surface energy, surface chemistry and topography.

In the present work, a model for bacterial adhesion is introduced that describes the early stages of biofilm formation as a function of the surface properties. A two-dimensional Cellular Automaton (CA) / Finite Difference (FD) adsorption model is combined with the predictions of the extended DLVO (Derjaguin, Landau, Verwey, Overbeek) theory that accounts for the interaction energies between the bacteria and the material's surface. The model describes the mass transport of bacteria in an aqueous solution towards the material's surface and the adsorption and desorption process, depending on the surface properties.

The adhesion process of different human pathogenic bacteria (*Enterococcus faecalis, Staphylococcus aureus, Escherichia coli*) on different biomaterial surfaces (titanium, stainless steel, polyethylene, polymethylmethacrylate, polytetrafluoroethylene) has been simulated. Results are the surface coverage with bacteria and, where applicable, clustering of the bacteria due to their migration on the surface.

Excellent agreement with experimental findings from the literature and own adhesion experiments concerning the kinetics of the adsorption process is found. In addition, a realistic bond strengthening mechanism of bacteria on surfaces, as described in the literature, is reproduced by the model. By using a spatial pattern analysis of our own experimental data we show that physical processes occurring during initial stages of the adhesion process are essentially correctly incorporated in the model.

8:40am **BI-WeM3 A Library of Polymer Gradients for Understanding Bacteria-Material Interactions**, ***A.L. Hook***, *J. Yang, C.-Y. Chang,* University of Nottingham, UK, *D.G. Anderson, R. Langer,* Massachusetts Institute of Technology, *S. Atkinson, P. Williams, M.C. Davies, M.R. Alexander*, University of Nottingham, UK

Biofilm formation leads to a 1000 times increase in antibiotic tolerance compared with planktonic bacteria and is associated with 80% of hospital acquired infections, resulting in $3.0 billion in excess health-care costs each year in the U.S alone. Thus, new materials for biomedical devices that prevent biofilm formation would offer enormous benefits to the health industry and improve patient welfare. However, our current understanding of bacteria-material interactions limits scope for rational design of such materials. Polymer microarrays are emerging as a key enabling technology for the discovery of new biomaterials.[1] A method for forming polymer microarrays has been developed using contact printing to deposit nanolitre volumes of premixed acrylate monomer and initiator to defined locations on a poly(HEMA) coated glass slide with UV photo-initiation.[2] This platform enables a large combinatorial space to be rapidly screened by a biological assay to identify new materials that fulfil a given performance criterion.[3] A library of polymer gradients that enables the systematic investigation of biology-material interactions can be created by producing polymers from monomers mixed at hundreds of different concentrations. Utilising a high throughput surface characterisation approach the surface chemical and physical properties of each material can be characterised and related to the biological performance.[4] We have developed a high throughput bacterial attachment assay based on three pathogens (*Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli)* expressing green fluorescent protein, which is compatible with the polymer microarray format. This study provides novel insights into the bacteria-material interactions, highlighting chemical moieties that both support and resist bacterial attachment. Specifically, superior efficiacy to prevent bacterial attachment has been demonstrated for hydrophobic moieties on a polyacrylate backbone that contains weakly polar ester groups, which represent an amphiphillic chemical nature.

[1] Hook, A. L. et al., High throughput methods applied in biomaterial development and discovery. *Biomaterials* **31** (2), 187 (2010).

[2] Anderson, D. G., Levenberg, S., and Langer, R., Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells. *Nature Biotechnology* **22** (7), 863 (2004).

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9:00am **BI-WeM4 Developing Tools to Observe Microbial Metabolic Exchange in 2D and 3D**, *J. Watrous,* University of California, San Diego, *T. Alexandrov,* University of Bremen, Germany, *W.-T. Liu, A. Lamsa, D. Gonzalez, N. Bandeira, M. Hamby, R. Kersten, K. Pogliano, B. Moore,* ***P.C. Dorrestein***, University of California, San Diego **INVITED**

From the early days of bacterial culturing over a century ago, microbiologists have known that microorganisms respond to their surroundings. Unicellular organisms rely on natural product mediated metabolic exchange to adapt to environmental stresses, sense colony density, and form biofilms. However, studies of the chemistry and phenotypes that correspond to signaling behavior have largely been disconnected and measured indirectly. To connect the chemistry and phenotypes, imaging mass spectrometry (IMS) methodologies are developed to observe metabolic exchange mediated within pair-wise interactions and microbial communities in two- and three dimensions. IMS provides the ability to correlate the presence of metabolites to phenotypic changes and to detect new biological phenotypes. Many of such phenotypes cannot be observed by the naked eye.

9:40am **BI-WeM6 Analysis of Cancer Cell Lines with ToF-SIMS and PCA**, ***M. Robinson***, University of Washington, *F. Morrish, D. Hockenberry,* Fred Hutchinson Cancer Research Center, *L.J. Gamble*, University of Washington

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) has been increasingly utilized for examining biological samples including biomaterials, cells, and tissues. The primary advantage of this MS technique is that it produces a chemical map of a sample, which includes hundreds of peaks that are detected in parallel. The advent of cluster ion sources has allowed the detection of many high mass lipid species that can be used to characterize biological surfaces [1]. In conjunction with principal component analysis (PCA), we use ToF-SIMS to determine differences in the chemical makeup of the outer membrane of two different cancer cell lines: MDA-231 and MCF-7 cells. There is similar work currently being done that uses Ultra Performance Liquid Chromatography-MS and gene sequencing to start characterizing lipid membrane metabolism in breast cancer tumor tissue [2]. The separation of the two cell lines across PC1 can be clearly seen in Figure 1. The entirety of the loads for PC1 can be seen in Figure 2, with cholesterol strongly loading towards the MDA-231 cells and many diacylglycerol (DAG) species loading towards the MCF-7 cells. Key differences were found in the levels of certain lipid constituents of the cell membrane, which may play a role in the ability of one cell type to be more drug resistant than the other. There are a variety of lipid components that have similar trends which are not discussed in this abstract but may play an important role in understanding this system.

This work is the foundation for future studies using human tumor biopsy samples that will help elucidate the link between fatty acid composition within a tumor and the potential drug resistance of that tumor.

10:40am **BI-WeM9 Engineering Stem Cell Differentiation via Material Properties**, ***T. McDevitt***, Georgia Institute of Technology **INVITED**

Stem cell differentiation is sensitive to a variety of global and local environmental cues that impact cell fate decisions. Pluripotent stem cells (i.e. ESCs & iPSCs) are capable of recapitulating many aspects of early development and can serve as a robust cell source for the development of cell-based diagnostics and regenerative medicine therapies. ESCs are commonly differentiated as three-dimensional multi-cellular aggregates referred to as "embryoid bodies" (EBs), because of their ability to mimic the early morphogenic transformation of pluripotent cells into derivatives of the three germ lineages (ecto-, endo-, and mesoderm). In order to better understand and ultimately control ESC morphogenesis, w e have focused on systematically engineering biochemical and biophysical parameters of the 3D EB microenvironment via the integration of different biomaterials and examining the emergent results on stem cell differentiation. Microparticles (MPs) of varying size (1-20 μm) and chemistries (i.e. PLGA, agarose, gelatin) were incorporated within 3D stem cell aggregates in a dose-dependent manner (~1 particle / 10 cells) without adversely affecting cell viability. Interestingly, the mere presence of relatively small numbers of different types of materials alone could modulate stem cell phenotype as evidenced by gene expression profiling and immunophenotype analyses. D elivery of morphogenic factors, such as retinoic acid (RA), bone morphogenic protein 4 (BMP4) or vascular endothelial growth factor (VEGF), to ESCs from incorporated MPs significantly impacted the differentiation of the cells to different lineages and was more efficient than comparable soluble treatment methods. Altogether these results suggest that engineered biomaterials can direct the differentiation of stem cells through modulation of biochemical and/or biophysical properties of the 3D microenvironment. It is expected that the development of inherently scalable techniques to direct pluripotent stem cell differentiation will benefit

the biomanufacturing of stem cell derivatives for regenerative cellular therapies and *in vitro* cell based diagnostic technologies, as well as enable engineering of tissues directly from stem cells.

11:20am **BI-WeM11 Adhesion and Rolling of Leukemic Cells on Immobilized Hyaluron**s, ***A. Rosenhahn***, Karlsruhe Institute of Technology, Germany, *C. Christophis, I. Taubert, G.R. Meseck, A.D. Ho, M. Grunze*, University of Heidelberg, Germany

Adhesion and rolling on vessel walls are two processes which are relevant for the homing of hematopoietic cells. Especially in the case of acute leukemia, one key in successful therapy is the homing of the hematopoietic stem cells (HSC) to the bone marrow after transplantation. We investigated the interaction of HSC with the hyaluron binding motive and quantitatively studied the interaction of different leukemic cells with synthetic polysaccharide surfaces. For the experiments we applied a microfluidic shear force assay recently developed in our group [1]. Leukemic Jurkat and Kasumi-1 cells lacking CD44-expression showed no adhesion or rolling on the polysaccharides whereas CD44 expressing leukemic cells KG-1a, HL-60, and K-562 attached and rolled on hyaluronan. We find that at weak flow cells have a poor tendency to adhere and only if shear forces above a treshold are present, adhesion is mediated. While this effect is well known for leukocytes on hyaluronan expressing feeder layers, it is the first demonstration that the mechanism also occurs in leukemic progenitor cells towards synthetic hyaluronan coated surfaces. We also extended the study to hematopoietic progenitor cells and saw for the first time that also HPCs with high degree of stemness show a flow induced interaction with hyalurons.

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Helium Ion Microscopy Focus Topic
Room: 106 - Session HI+AS+BI+NS-WeM

Nano- and Bio- Imaging with Helium Ion Microscopy

Moderator: A. Gölzhäuser, University of Bielefeld, Germany, V.S. Smentkowski, GE-GRC

8:40am **HI+AS+BI+NS-WeM3 Helium Ion Microscopy Techniques for Imaging and Characterization of nano-Device Materials and Structures**, ***S. Ogawa***, *T. Iijima*, National Institute of Advanced Industrial Science and Technology (AIST), Japan **INVITED**

This paper presents several imaging modes for nano-devices fabrication that may make HIM a tool of particular value to soft materials such as low-k dielectrics (low-k) with less transformation and more materials contrast which reflects damaged areas, and copper interconnect buried in dielectrics, and shows luminescence induced by the focused helium ion beam using the HIM for the first time.

Imaging of -100 nm pitch patterned low-k is important for LSI Cu/low-k interconnect processes, while SEM imaging results in changes to the low-k line edge roughness and shape by damage during an electron beam irradiation. The HIM could provide low-k dielectric secondary electron (SE) image with nm order resolution, deeper focus depth, less transformation because of three order magnitude lower thermal energy transfer into a unit volume of the low-k than the SEM under an appropriate operation condition[1].

During the imaging, even at very low helium ion current, surfaces of samples were atomically etched off, as in a graphene patterning, and then blistering or physical etch occurred with the increase of the helium ion current. This makes the interpretation of the HIM SE imaging difficult but helpful. Damaged areas at side walls of the low-k regions in a 140 nm pitch interconnect were successfully seen with a different contrast from non-damaged low-k regions at an "optimized" helium ion beam condition[2], which was similar to a TEM/Valence EELS result. On the other hand, using the SEM, the damaged areas contrast in the low-k regions could not been imaged.

A new imaging mode, through the inter-level dielectric, of the underlying copper, was explored. Cu interconnect was seen through a 130 nm thick low-k dielectrics. The incident helium ions might generate secondary electrons(SEs) at the buried Cu surface and the SEs of 1-2 eV energy passed through the dielectric of a few eV band gap without any energy transfer, and then the image was obtained. Helium ion channeling at the Cu surface area varied the secondary electron quantity, and it might generate a crystal orientation contrast of the buried Cu metal.

Luminescence induced by the focused helium ion beam was studied using the HIM[2]. Helium ion beam of a few pA current was irradiated to a SiO_2 film, and peaks in a spectrum were observed at around 281, 447, and 672 nm; these positions were different from those by a conventional SEM cathode luminescence. The further study will be presented.

L.Stern, W.Thompson and J.Nottte of Carl Zeiss are acknowledged for their discussions in the Cu / low-k works.

1) S. Ogawa, et al, Jpn. J. Appl. Phys., 49 (2010) 04DB12, 2) S. Ogawa, et al, Proc. of 2011 IEEE IITC (2011)

9:20am **HI+AS+BI+NS-WeM5 He Ions Image the Au (111) Herringbone Reconstruction**, ***V. Veligura***, *G. Hlawacek, R. van Gastel, H. Zandvliet, B. Poelsema*, MESA+ Institute for Nanotechnology, University of Twente, Enschede, The Netherlands

The herringbone reconstruction of the Au(111) surface was directly visualized using an Ultra High Vacuum Helium Ion Microscope. Ion channeling phenomena arise from the different atomic ordering in the outermost layer of the crystal. First, we investigated the channeling contrast from the bulk Au fcc structure by imaging polycrystalline Au on glass films. The contrast that was observed as a function of crystal orientation was found to conform to what is calculated from a simple hard sphere model. Consequently, the herringbone reconstruction was investigated. It is a periodic zigzag structure of the three different types of crystal stacking (fcc, hcp and bridge sites connecting these regions) and, ideally, has a period of 6.3 nm. The different stacking of the atoms that constitute the surface reconstruction leads to lateral variations of the secondary electron yield that can be resolved in HIM imagery. The existence of the herringbone reconstruction on the sample was independently confirmed through STM measurements and the quantitative details from both techniques are similar, but seem to be affected by the differences in vacuum conditions. An influence of both the ion beam and vacuum environment on the visibility of the herringbone reconstruction is observed in our UHV-HIM system.

9:40am **HI+AS+BI+NS-WeM6 Imaging of Graphenoid Nanomembranes with Helium-Ion Microscopy**, ***A. Beyer***, *A. Turchanin, A. Gölzhäuser*, University of Bielefeld, Germany

Helium-ion microscopy is known for its high surface sensitivity. Here we present a study about imaging extremely thin nano-scale objects: graphenoid nanomembranes which consist exclusively of atoms near the surface. Such freestanding nanomembranes with a thickness of 1 nm are made from self-assembled monolayers (SAMs) by cross-linking and subsequent transfer to transmission electron microscopy (TEM) grids or other suitable substrates. We show that these nanomembranes exhibit a substantially higher contrast in helium-ion microscopes as compared to electron microscopes.

Cross-linking of SAMs is performed by large area exposures with electrons or photons which yield extended nanomembranes. On the other hand, patterned exposures allow the fabrication of nanosieves, i.e. perforated nanomembranes. Advantages in imaging such patterned cross-linked SAMs as well as freestanding nanosieves with the helium-ion microscope will be discussed.

10:40am **HI+AS+BI+NS-WeM9 Nanofabrication and Biological Imaging with the Helium Ion Microscope**, ***D.S. Pickard***, National University of Singapore **INVITED**

The Helium Ion Microscope (HIM) is a new imaging technology based on a high brightness and stable Gas Field Ion Source (GFIS). The GFIS employed exhibits a low energy spread (<1 eV), small virtual source size (< 0.3 nm) and a high brightness > 4 x 10^9 A/cm^2.sr [1]. This, in conjunction with the shallow escape depth (<1 nm) of the secondary electrons generated by the incident 30 keV helium ions, contribute to the HIM's primary advantage in the imaging of solid samples: its high spatial resolution (0.25 nm) [2]. We have applied this novel technology across a broad spectrum of multidisciplinary applications (from basic materials science and semiconductor applications to the biological sciences) to assess its utility and possible advantages over alternative techniques.

One area where our investigations have gained significant traction is in the imaging of biological specimens. The utility of this instrument in addressing topics of the biological sciences is due in part to the HIM's high spatial resolution. However, in the context of biological specimens, it is the ability to image non-conductive samples without the application of a metal (or other conductive) overcoat and without the need of a background gas (both of which degrade resolution and surface details), which has proven to be a distinguishing attribute. This opens up a whole new range of biological problems that can be solved rapidly and with less risk of artifacts.

An equally compelling application is in the field of nano-structuring. The focused helium ions have the ability to directly modify the sample surface under a high ion flux (via surface sputtering). This enables the direct patterning of structures and promises great utility in the fabrication of sub-

10 nm devices. It also provides a mechanism for high resolution patterning on nonconventional substrates (such as suspended graphene membranes), where resist-based lithographic techniques are not feasible. Our experiences in sub-10 nm pattern transfer for both graphene and plasmonics applications will be presented.

1. B. Ward, J. Notte, and N. Economou, J. Vac. Sci. Technol. B, Vol. 24, No. 6, Nov/Dec 2006

2. Application Note, Carl Zeiss SMT, "Ultra-High Resolution Imaging in ORION®PLUS", PI No. 0220-2008-ENG, Nov. 21, 2008

11:20am **HI+AS+BI+NS-WeM11 Imaging and Characterizing Cellular Interaction of Nanoparticles using Helium Ion Microscopy**, ***B.W. Arey***, *V. Shutthanandan, Y. Xie, A. Tolic, G. Orr*, Pacific Northwest National Laboratory

The helium ion mircroscope (HeIM) probes light elements (e.g. C, N, O, P) with high contrast due to the large variation in secondary electron yield, which minimizes the necessity of specimen staining. A defining characteristic of HIM is its remarkable capability to neutralize charge by the implementation of an electron flood gun, which eliminates the need for coating non-conductive specimens for imaging at high resolution. In addition, the small convergence angle in HeIM offers a large depth of field (~5x FE-SEM), enabling tall structures to be viewed in focus within a single image. Taking advantage of these capabilities, we investigate the interactions of engineered nanoparticles (NPs) at the surface of alveolar type II epithelial cells grown in culture. The increasing use of nanomaterials in a wide range of commercial applications has the potential to increase human exposure to these materials, but the impact of such exposure on human health is still unclear. One of the main routs of exposure is the respiratory tract, where alveolar epithelial cells present a vulnerable target. Since the cellular interactions of NPs govern the cellular response and ultimately determine the impact on human health, our studies will help delineating relationships between particle properties and cellular interactions and response to better evaluate NP toxicity or biocompatibility.

The Rutherford backscattered ion (RBI) is a helium ions imaging mode, which backscatters helium ions from every element except hydrogen, with a backscatter yield that depends on the atomic number of the target. Energy-sensitive backscatter analysis is being developed, which when combined with RBI image information, support elemental identification at helium ion submicron resolution. This capability will enable distinguishing NPs from cell surface structures with nanometer resolution.

11:40am **HI+AS+BI+NS-WeM12 Application of Helium Ion Microscope on Semiconductor Surface Imaging and Metrology**, ***H.X. Guo***, National Institute for Materials Science, Japan, *H. Itoh,* National Institute of Advanced Industrial Science and Technology (AIST), Japan, *K. Onishi, T. Iwasaki, D. Fujita*, National Institute for Materials Science, Japan

Scanning electron microscope (SEM) has been used in the semiconductor surface imaging and metrology for more than 50 years. Now, a new tool, Helium ion microscope (HeIM), is developed and applied to this work. SEM and HeIM are the same in some fundamental characteristics. But, the latter has advantages in smaller probe size, higher resolution, and greater depth of field. These abilities enhance the performance of the HeIM in the semiconductor surface imaging and metrology, such as imaging of low–k materials [1] and measurement of critical dimension of the semiconductor devices [2].

A standard sample for scanning probe microscope tip characterization [3, 4] was measured by using HeIM and atomic force microscope (AFM) as shown in Fig. 1 and Fig. 2. Line profile of the HeIM image in Fig. 1 shows high accuracy in edge definition of the sample. The contrast of the image is related to morphology and materials of the sample [5], the probe size of the Helium ion beam, direction of the sample and beam, charge distribution, and so on. All the aspects will be analyzed in our presentation. The AFM image of the sample shown in Fig. 2 is a dilation of the real surface topography of the sample due to the finite-size AFM tip [6]. With an erosion algorithm, the surface of the sample was reconstructed to be compared with HeIM measurement.

[1] S. Ogawa, W. Thompson, L. Stern, L. Scipioni, J. Notte, L. Farkas , and L. Barriss, Jpn. J. Appl. Phys., 49, 04DB12(2010)

[2] M. T Postek , A. Vladar , C. Archie and B. Ming, Meas. Sci. Technol., 22, 024004 (2011)

[3] H. Itoh, C. Wang, H. Takagi, Proc. of SPIE, 7971, 79711A-1, (2011).

[4] H. Takenaka, M. Hatayama, H. Ito, T. Ohchi, A. Takano, S. Kurosawa, H. Itoh, and S. Ichimura, Journal of Surface Analysis, 17, 264, (2011).

[5] Y. Sakai, T. Yamada, T. Suzuki, T. Sato, H. Itoh, and T. Ichinokawa, Appl. Phys. Lett., 73, 611 (1998)

[6] M. Xu, D. Fujita, and K. Onishi, Rev. Sci. Instrum., 80, 043703 (2009)

Biomaterial Interfaces Division
Room: 108 - Session BI+AS+NS+SS-WeA

Functionalization and Characterization of Nanostructures

Moderator: A. Belu, Medtronic, Inc.

2:00pm **BI+AS+NS+SS-WeA1 Characterization of Nano-objects by Cluster-SIMS**, ***E.A. Schweikert***, Texas A&M University **INVITED**

Secondary ion mass spectrometry, SIMS is a method of choice for the chemical analysis of nanodomains embedded in solids. We examine here a case which has received little attention, the analysis of individual, free-standing nano-objects. Our approach uses a variant of SIMS. The nano-objects are bombarded with a sequence of individual projectiles resolved in time and space, in the present case Au_{400}^{4+} of up to 520 keV impact energy. The successive projectiles impact stochastically the nano-objects dispersed on a solid support. Typically tens of secondary ions are ejected from each impact. They are identified with time-of-flight mass spectrometry and recorded individually. This approach reveals molecules co-located within the 10-20 nm diameter area of emission from one Au_{400}^{4+} impact. We demonstrate that the event-by-event bombardment-detection mode is sensitive to the chemical and/or physical nano-scale separation of molecular species. The performance is illustrated with the determination of the relative abundance of the oxide layer in the near surface of 50-100 nm nanoparticles; the nature and abundance of different nano-objects (5-20 nm in diameter) in mixtures of nano-sized solids; the composition of bio-objects such as a bacteriophage including the amino acids of the proteins surrounding the phage and the bases from the encapsulated DNA. The distinct feature of the nanoprobe technique presented here is in the detection of co-emitted ejecta from individual projectile impacts which allows to test chemical composition, in a nonimaging mode, yet at an exquisite level of spatial resolution. Moreover the co-emission of fragment and parent ions enhances the accuracy of molecular identification.

Work supported by NSF grant CHE-0750377

2:40pm **BI+AS+NS+SS-WeA3 Strategies for Studying the Surface Chemistry of Engineered Nanoparticles with SIMS**, ***C. Szakal***, *J. McCarthy,* National Institute of Standards and Technology*, K. Louis, R.J. Hamers,* University of Wisconsin-Madison*, R.D. Holbrook*, National Institute of Standards and Technology

The environmental toxicity of engineered nanoparticles (ENPs) is of increasing importance as these materials become more widely used in manufacturing processes and consumer products. Nanoparticles have extremely high surface-to-volume ratios, which makes the surfaces more critical than their corresponding bulk materials in terms of reactivity, aggregation, and toxicity to various life forms. Therefore, it is critical that we develop methods to distinguish small chemical changes on nanoparticle surfaces in order to understand how these materials will interact outside of controlled laboratories. Conventional approaches of nanoparticle characterization have focused on high resolution morphological imaging (TEM, SEM) and physical property measurements such as surface charge. However, chemical information is generally only inferred from these materials with most current methods. If it is possible to obtain both elemental and molecular information from ENP surfaces, we may be able to determine the eventual fate of ENPs in the environment.

We have developed a comprehensive approach for studying the surface chemistry of ENPs, including 1) preparation of ENPs to controllably study desired variables, 2) development of methods such as time-of-flight secondary ion mass spectrometry (ToF-SIMS) and environmental scanning electron microscopy (ESEM) to probe small changes in ENP surface chemistry and/or aggregation, and 3) development of methods to improve the speed and reproducibility of ENP aggregation for batch studies. These approaches will be utilized as the basis of future toxicity studies of selected ecosystems.

3:00pm **BI+AS+NS+SS-WeA4 Unusual Hydrogenation Isotherms for Pd Nanoring Model Systems Observed Via Nanoplasmonic Sensing**, *C.B. Langhammer, E.M.K. Larsson,* ***I.L. Zoric***, Chalmers University of Technology, Sweden*, V.P. Zhdanov*, Boreskov Institute of Catalysis, Russian Federation

Nanostructured materials have been proposed as a solution for the development of efficient hydrogen storage systems. As the size of the system gets reduced in the nanometer range enthalpies and entropies of hydrogen dissolution in the metal (α-phase) and hydride formation (β-phase) as well as activation barriers for diffusion and desorption of hydrogen become size dependent thus influencing both thermodynamics (pressure-composition isotherms) and kinetics (loading/unloading kinetics). The pressure-composition isotherms for a H2/M system show a well-known behavior typical for an α-phase in the low pressure-composition range followed by a plateau signaling the onset of a hydride formation (β-phase) via a first order phase transition and a coexistence of the two over a wide composition range. At higher pressure a pure β-phase exists characterized by a pressure-composition curve with a steep slope. The plateau pressure of the H2/M system is determined by the requirement of equilibrium between the three phases in coexistence, thus primarily by the enthalpy and entropy of hydride formation. In case of more than one hydride type a coexistence region exhibits multiple plateaux determined by appropriate energetics as described above. In all known cases the same multiple plateaux features were observed both during the charging and discharging process, i.e. when hydrogen pressure was increased/decreased, accompanied of course by a perennial hysteresis.

In this work we report unusual pressure-composition isotherms for H2/Pd nanosized rings where a double plateau isotherms are observed during the charging process and a single plateau one during the hydrogen discharging. The Pd nanorings were fabricated using colloidal lithography. Hydrogen isotherms were followed by monitoring the shift in the ring Localized Surface Plasmon Resonance, LSPR, upon exposing the system to increasing/decreasing hydrogen pressure steps (and equilibrating the sample at each pressure step). The shift of the LSPR resonance was calibrated by preparing the same ring structure on a quartz crystal microbalance and "weighing" directly absorbed hydrogen. A linear relation between the LSPR shift and QCM frequency shift (proportional to hydrogen concentration) was observed.

We present a simple model, based on the observed heterogeneity of the nanorings (as seen in TEM) and by taking into account the defect induced lattice strain, that accounts for the unusual behavior of the observed isotherms.

4:00pm **BI+AS+NS+SS-WeA7 Surface Functionalization and Analysis of Functional "Soft" Nanostructures: From 2 to 3 Dimensions**, ***H. Schönherr***, University of Siegen, Germany **INVITED**

The local properties of soft matter, e.g. for the fabrication of functional biointerfaces or nanostructures, are of tremendous importance for ultimate functionality. In this presentation, the closely interrelated areas of surface chemical functionalization / engineering and analysis of properties will be discussed based on three key examples. These example include: (i) synthesis and modification of polymer brushes with particular focus on the nanomechanical properties, (ii) ultra small diameter nanotubes obtained by the layer-by-layer assembly of polyelectrolytes inside a sacrificial porous template and (iii) block copolymer nanocapsules that are developed for advanced wound management. In all examples, confinement effects are expected to play a significant role in determining e.g. the mechanical properties, as assessed by atomic force microscopy (AFM) nanoindentation.

For thin polymer films (2D) the dependence of the mechanical properties on the film architecture was unraveled. Compared to spin-coated films, brushes synthesized on gold surface by surface initiated polymerization showed higher elastic moduli, which is attributed to entropy effects. Upon chemical crosslinking tunable elastic properties are obtained, which provides interesting pathways for the fabrication of defined cell - surface contacts.

Similarly important are defined nanoscale objects that can be obtained via the replication of small templates by the so-called layer by layer (LbL) deposition of polyelectrolytes (G. Decher Science 1997, 277, 1232). LbL deposition in porous Anodic Aluminum Oxide (AAO) was only very recently expanded to the 100 nm length scale due to an alleged entropic barrier caused by adsorbed polyelectrolytes close to the pore orifice [Y. Cho et al. Small 2010, 6, 23, 2683.]. However, in contrast to this report, we show that the adsorption of polelectrolytes on the top plane of the AAO and polymer sedimentation have been identified as main bottlenecks. Suppressing these processes enabled us to produce free standing polymer nanotubes with external diameters of < 55 nm.

Finally, first steps in the development of active nanocapsules filled with a reporter dye or an antimicrobial agent for applications in burn wound management will be presented. In particular the case of burn wounds and the devised biomimetic strategy of BacterioSafe will be introduced. Subsequently, the fabrication and characterization of a polystyrene-block-poly(acrylic acid) amphiphile-based model vesicle system, in particular the loading and release behavior and mechanical properties will be discussed.

4:40pm **BI+AS+NS+SS-WeA9 Large Area Fabrication of Biological Nanostructures**, *G. Tizazu, O. El-Zubir,* University of Sheffield, UK*, S. Brueck,* University of New Mexico*, D. Lidzey,* ***G. Leggett****,* University of Sheffield, UK*, G.P. Lopez*, Duke University

There has been enormous interest in the control of biological interactions at interfaces with nanometer spatial resolution, but important challenges still remain to be addressed. Of the established fabrication techniques, electron beam lithography is expensive, and requires exposure under vacuum, while scanning probe methods are slow and (with few exceptions) do not permit fabrication over large areas. In contrast, interferometric lithography (IL) is a simple approach that uses inexpensive apparatus to fabricate sub-wavelength structures over macroscopic areas. When two coherent laser beams interfere, they yield a sinusoidal pattern of intensity that may be used to modify photosensitive materials. Previously IL has been used extensively for semiconductor nanofabrication, but our recent data show that combined with self-assembled monolayer resists it provides a fast, simple method to create molecular nanostructures over macroscopic areas. Illustrations will be provided of bionanofabrication using interferometric modification of protein-resistant (oligo ethylene glycol) functionalised surfaces, where feature sizes as small as 30 nm (λ/8) have been achieved over square cm areas, and the controlled growth of protein-resistant brush structures from patterns of initiators for atom-transfer radical polymerisation. The fabrication of metallic nanostructures over macroscopic regions, including Ti structures as small as 35 nm, and gold nanostructures of controlled size and periodicity will also be demonstrated.

5:00pm **BI+AS+NS+SS-WeA10 Functionalization of Mesoporous Silicon Biosensors to Achieve Tunable DNA Bioreceptor Density**, ***J. Lawrie****, R.R. Harl, B.R. Rogers, P. Laibinis, S.M. Weiss*, Vanderbilt University

Porous silicon has become a widely studied material for sensing over the last decade based on its large surface to volume ratio and easily tunable morphology. With growing interest in the detection and analysis of genetic material, DNA oligos have become an increasingly important bio-recognition element in porous silicon and many other sensor platforms. As aptamers, nucleic acids serve as high affinity bioreceptors to a wide range of small molecules and biological materials, opening up a number of potential applications in environmental science, chemical and biological defense, and medical diagnostics. In this work, tuning of the porous silicon surface chemistry is described. Controlling surface silanization, bioreceptor density, and bioreceptor charge and secondary structure enables the fabrication of reusable, label-free optical sensors toward specific nucleic acid targets. Detection limits in the nanomolar range have been demonstrated.

We have previously shown that in situ DNA synthesis via the phosphoramidite method in porous silicon produces high bioreceptor coverage for label-free optical biosensing applications. Low hybridization efficiency, despite high sensitivity, for such sensors indicated that tuning the receptor surface density could further improve detection limits. To modify surface receptor density, two-component trichlorosilane monolayers were deposited from solution onto porous silicon. One monolayer component remained active to phosphoramidite chemistry while the second component was inert. This method enabled a range of surface probe densities to be achieved and controlled via silanization conditions. Monolayer composition and DNA receptor density were verified using XPS, contact angle, and UV-Vis spectrophotometry. For a 16mer DNA oligo bound within a porous silicon waveguide, detection of the complementary target nucleic acid was maximized when 25% of the internal pore surface area was active toward DNA synthesis. Tuning surface DNA density increases sensitivity by a factor of 2-3. Label-free, target-specific detection of oligos was observed at concentrations of 25nM.

We will present results from hybridization efficiency studies in which DNA bioreceptor surface density, length, and secondary structure are varied. These parameters are vital to nucleic acid aptamer sensing strategies in label-free optical biosensors. Predicting appropriate receptor surface density for aptamer sensors based upon oligo sequences will provide advantages in achieving fast and sensitive waveguide sensors for detection in complex media.

Acknowledgements: This work is supported in part by the Army Research Office (W911NF-08-1-0200).

5:20pm **BI+AS+NS+SS-WeA11 Composite Fluorocarbon Membranes by Surface-Initiated Polymerization**, ***C.A. Escobar****, A.R. Zulkifli, G.K. Jennings*, Vanderbilt University

This presentation describes the fabrication and characterization of a novel composite membrane that consists of two types of nanoporous materials, namely, nanoporous gold leaf (NPGL) and nanoporous alumina, and a selective poly(perfluorohexyl norbornene) (pNBF6) polymer. Integration of the three materials is achieved by means of silane and thiol chemistry, and the use of surface-initiated ring-opening metathesis polymerization (SI–ROMP). The former two provide functionalization of the nanoporous substrates, and the latter promotes the generation of the polymer film within and atop of the alumina-NPGL membrane. The synthetic process is versatile in that simultaneous or selective growth of the polymer film in each nanoporous layer is straightforward. The use of SI–ROMP allows tailoring of the extent of polymerization of pNBF6 throughout the structure by varying polymerization time. Advancing contact angle measurements show that the surface of these composite membranes exhibits both hydrophobic and oleophobic behavior. Scanning electron microscopy (SEM) images indicate that the thin polymer films cover the porous substrates entirely. Results from electrochemical impedance spectroscopy (EIS) confirm that the membranes provide effective barriers to aqueous ions and that sulfonation of the polymer backbone substantially enhances ion transport through the composite membrane. Ion transport and selectivity of the membrane change by regulating the polymerization time. The fluorinated nature of the polymer thin film renders the membrane selective towards molecules with similar chemical characteristics.

Biomaterial Interfaces Division
Room: 108 - Session BI-ThM

Biomedical Materials
Moderator: S.L. McArthur, Swinburne University of Technology, Australia

8:20am **BI-ThM2 Surface Characterization: A Critical Component in Understanding the Biocompatibility of Biomaterials**, ***L. Salvati***, *S. Vass*, DePuy Orthopaedics **INVITED**

A biomaterial is defined as "any synthetic material or device–e.g. implant or prosthesis-intended to treat, enhance or replace an aging or malfunctioning native tissue, organ or function in the body" David Williams, states that ***"Biocompatibility is largely about the chemical interactions that take place between the materials and the body fluids, and the physiological responses to these reactions."*** These reactions are dominated by the initial *events at* the molecular level, the interface, thus it is not hard to see the relationship between the implant surface properties and it's in vivo. It is clear that the performance of a biomaterial is directly linked to the surface chemistry, composition and topography of the device. However, despite the preponderance of evidence, biomedical device companies as a whole do not utilize surafce analytical methods nearly enough. Why is that?

In orthopaedics, most of the implants are fabricated from metals which directly contact biological fluids that are typically complex aqueous mixtures. Consequently corrosion and/or corrosion prevention are important considerations in device manufacturing. For the most part, orthopaedics manufacturers utilize the same passivation methodologies used to impart corrosion resistance to stainless materials. Numerous researchers have characterized the impact of acid passivation on stainless materials, but little has been done to study the effect of these treatments on the CoCr alloys. One of the topics in this presentation deals with the effects of passivation on CoCr alloys. Specifically, the presentation will correlate specific surface treatments with surface chemistry and ultimately to metal ion release. The study will also address questions relating to the use of Citric acid as a green replacement for nitric acid passivation. There is no argument that the process is much greener, but there are plenty of questions as to its effectiveness. Considering that the "changes" imparted by the passivation solutions effect the outer most 10-100A, the only means to characterize the affects of passivation requires surface analysis methodologies. The importance of surface analysis methods, especially XPS, will be highlighted by this example.

In addition to the discussion of metal alloy passivation, this presentation will also deal with other surface related issues that could impact the biocompatibility of biomaterials. The talk will show the potential impact of packaging materials and cleaning processes on the surface chemistry and composition of biomaterials.

9:00am **BI-ThM4 XPS Sputter Depth Profiling of Organic Materials Using a Coronene Ion Source**, ***S.J. Hutton***, *C.J. Blomfield, A.J. Roberts, S.C. Page, S.J. Coultas,* Kratos Analytical Ltd, UK, *C.E. Moffitt, D.J. Surman*, Kratos Analytical Inc

Controlled release of active pharmaceutical molecules from biocompatible polymers over defined time periods is an area of intense study. Present applications include drug eluting stents and other drug delivery systems. One of the most important parameters which govern drug dosing is the drug concentration depth profile in the supporting polymer matrix. In a previous study we have shown that combining X-ray photoelectron spectroscopy (XPS) with a coronene ion source is a very powerful tool for investigating the drug distribution with depth of a model system [1].

The use of cluster ion sources for sputter depth profiling of thin film or multilayer organic materials during XPS analysis has become routine. A wide range of organic systems are amenable to profiling and there is a good understanding of the experimental parameters which contribute to successful analysis. Here we report on extending the aforementioned study to materials which closely resemble real world samples intended for use in vivo.

[1] A. Rafati, M.C. Davies, A.G. Shard, S. Hutton, G. Mishra, M.R. Alexander, *J. Controlled Release*, **2009**, *138,* 40–44

9:40am **BI-ThM6 Amino-rich Plasma Polymer Films Prepared by RF Magnetron Sputtering**, *J. Hanuš, G. Ceccone,* ***F.J. Rossi***, European Commission, JRC. Institute for Health and Consumer Protection, Italy

RF magnetron sputtering of nylon 6.6 was used for the deposition of nitrogen rich films. Deposition was followed by N_2 H_2 plasma post-treatment to enhance primary amine concentration on the surface. Maximal reached NH_2 concentration was 11 % with aminoselectivity 13.5 %. The films exhibited small negative z-potential at basic pH with isoelectric point ~ pH 4.5. Bio properties of the films were tested by QCM in terms of ability to adsorb different proteins and their antigens. The interaction between the film and the buffer solution was also studied and compared to other films such as poly-acrylic acid and PEO plasma polymers. The results show that these films are stable and can be used as a platform where positively charged surfaces are needed

10:40am **BI-ThM9 Surface Analysis in Biotech & Pharma: A Surfeit of Frontiers**, ***E. Johnston***, Genzyme **INVITED**

Surface and interfacial analytical tools continue to provide new value and find unexpected new uses in the biotech and medical device industries. Some uses are investigational in nature and help solve critical problems within manufacturing and quality control. Other applications fall squarely within the realm of R&D - tilting the balance between feasibility of a product or obsolescence of a project, or providing fresh insight into the nature of biomaterial/biological interactions. By way of example, a study will be presented illustrating how TOF-SIMS was used to image a phosphate-binding drug particle in the complex matrix of the rat gastrointestinal tract. Sample preparation was challenging due to the highly hydrated nature of the tissue material. The results yielded surprising information about the ions that bind to this cationically charged particle and opened new avenues for inquiry and study.

11:20am **BI-ThM11 Enhancing Monoclonal Antibody Drug Detection by Developing a Microparticle-based Immunoassay**, ***N. Mendez***, *M.E. Ruidiaz, A.B. Sanchez, B.T. Messmer, A.C. Kummel*, University of California San Diego

Monoclonal antibodies are a notable and rising class of cancer therapeutics due to their enhanced targeting and immune system stimulation properties. Dosage guidelines are typically developed with many uncertainties which may affect treatment outcome and cause unwanted side effects. The requirement for an assay that can quickly and precisely measure the concentration of the monoclonal antibody in a serum sample of a patient during therapy is needed. The present study has demonstrated that the key to detection is compensation for variation in non-specific binding of serum to the assay surface. A microparticle-based assay with peptide antigen mimetics has been developed to rapidly determine the concentration of antibody drug present in serum specimens with high sensitivity. Alemtuzumab (anti-CD52) and rituximab (anti-CD20) antigen peptides, as discovered by phage display, were synthesized on 10 um TentaGel resin beads using conventional solid phase peptide synthesis techniques. The microparticle beads were modified to allow for multiplexing and microfluidic handling via fluorescent labeling and magnetic functionalization. The antigen-displaying fluoromagnetic particles were incubated with spiked serum samples which allowed free antibody to be captured. Primary antibody detection was performed on alemtuzumab while rituximab detection was used to compensate for non-specific serum binding to the beads. After washing, the beads were incubated with a fluorescently tagged secondary antibody for detection by flow cytometry. Serum from thirty (30) individual donors with various spiked serum concentrations of antibody drug were assessed using this assay. Analysis of bead fluorescence data allows for a limit of quantitation down to 0.5ug/ml of serum antibody drug concentration. Using detection of an antibody known to be absent in serum, an accurate compensation technique for non-specific binding has been developed on multifunctional antibody assay beads in realistic samples. The developed assay is robust against donor serum variation.

11:40am **BI-ThM12 Controlling the Hydroaffinity of Silicone/Hydrophobic Acrylic Surfaces of Intraocular Lenses using Visco-Elastic Colloids and Blood Proteins**, *N.X. Herbots,* ASU / SiO2 NanoTech Inc. / SiO2 Associates, LLC, ***R.J. Culbertson***, *Q.X. Bradley, D.A. Sell, A.M. Murphy,* Arizona State U., *C.H. Sell,* Arizona Vitro-Retinal Consultants, *H.M. Kwong,* Arizona Vitro-Retinal Consultants / ASU, *T. Kutz, A.S. Benitez, M.A. Hart, B.J. Wilkens, R.B. Bennett-Kennett*, Arizona State U.

Over 15 million cataract surgeries are performed each year world wide. 2-6 % of cataract patients suffer subsequently from diabetic and other retinal issues post surgery due to aging and accidents and must undergo a secondary eye surgery. Secondary surgery performed after implantation of artificial intra-ocular lenses (IOLs) can fail due to the fogging of IOL's from condensation of bodily fluids. New, high performance accommodating silicone and hydrophobic acrylic IOL's can fog during such surgery. This work solves the problem by modifying water affinity of IOL's using a polymer emulsion, VitreOx™ [1-5] with a 100% success rate in the lab. Ten

clinical trials yielded a success rate of 80% in the year 2010-211 with failure inferred to be due to blood proteins on IOL's.

Thus, the role of hydro-affinity of blood proteins preventing coagulation, heparin, present during surgery, has to be investigated. Our results show that heparin behaves identically to H2O on hydrophobic surfaces. Heparin simply de-wets on silicone IOL's and hydrophobic acrylic lenses. It does not prevent fogging on IOL's nor interfere with our anti-fogging emulsion.

Fibrinogen is the other protein investigated because it enhances blood coagulation and is often present in trauma situations. Fibrinogen applied to IOL's in various dilutions does prevent fogging. However, it cannot be removed after application on the IOL's, thus remaining as a potent coagulant agent in the eye. Thus fibrinogen can indeed prevent fogging, but is not viable since it cannot be removed after application like VitreOx™. Fribrinogen could explain why some IOL's fog while others do not during emergency secondary eye surgery.

[1] U. S. Patent Application “Molecular Films for Hydrophobic Implant Surfaces” N. Herbots, J. D. Bradley, M.A. Hart, D.A. Sell, S. D. Whaley, Q. Xing Bradley Filed 11/9/10

[2] "Modeling Mechanisms of Water Affinity & Condensation on Si-based Surfaces via Experiments & Applications" by Q. Xing, ASU (2011).

[3] N. Herbots, Q. Xing, M. Hart, J. D. Bradley, D. A. Sell, R. J. Culbertson, B. J. Wilkens; "IBMM of OH Adsorbates and Interphases on Si-based Materials" Nucl. Instr. & Meth. B, IBMM 17 (2010), accepted.

[4] Q. Xing, M. A. Hart, R. J. Culbertson, J. D. Bradley, N. Herbots, B. J. Wilkens, D. A. Sell, C. F. Watson;"Particle-Induced X-ray Emission of Silicate Coatings on High Impact Resistance Polycarbonates". 21st ICAARI (2010), accepted

[5] Q. Xing, N. Herbots, M. Hart, J. D. Bradley, B. J. Wilkens, D. A. Sell, C. H. Sell, H. M. Kwong, R. J. Culbertson, S. D. Whaley; "Ion Beam Analysis of Silicon-Based Surfaces and Correlation with Surface Energy Measurements?. 21st ICAARI (2010), accepted.

Biomaterial Interfaces Division
Room: East Exhibit Hall - Session BI-ThP

Biomaterial Interfaces Poster Session

BI-ThP1 Physicochemical Characterization of Loop Polymer Brushes Prepared by Immobilization of End-Functionalized Poly(dimethylsiloxane), ***S. Sakurai***, *H. Watanabe,* Jst, Erato, Japan*, A. Takahara*, Kyushu University, Japan

A loop polymer brush is surface-tethered polymer chain of which both ends were anchored on the substrate surface to form a loop at a molecular level. The dynamics and physicochemical characterization of the loop brush has been a hot topic of several theoretical and experimental studies for polymer physics because the formation and interaction of loop brushes are key processes in many important biological and engineering processes. In this study, we synthesized loop polymer brushes by using end-functionalized poly(dimethylsiloxane) (PDMS) with various molecular weights as follows. A commercially available amino-terminated PDMS was fractionated by size exclusion chromatography (SEC) in the molecular weight from M_n = 7,000 to 100,000 with narrow molecular weight distributions (M_w/M_n~1.2), and transformed to a triethoxysilane (TES) end-functionalized PDMS on both ends. The silicon wafer was immersed in dichloromethane solution of the telechelic TES-terminated PDMS at 293 K for 24 h to immobilize both chain ends of the PDMS. The surface morphology of the telechelic PDMS-immobilized silicon wafer was observed by atomic force microscopy (AFM) under air at room temperature. The root mean square (rms) of the surface roughness was 0.6 nm in a 2 x 2 μm^2 scanning area. The smooth morphology indicated that loop structure was formed without cross-linking and gelation of TES. Mono-functionalized PDMS was also synthesized to fabricate a linear brush on the silicon wafer. The thickness of these brushes in a dried state determined by ellipsometry increased from 2 to 5 nm with an increase in the molecular weight of M_n = 5,000 to 100,000. The loop and linear polymer brushes with same molecular weight showed the almost same thickness in a dried state. Apparent difference derived from a topology effect was not observed in a dried state. AFM measurement of the swollen thickness of the loop and linear brushes in a solution is in progress.

BI-ThP2 Surface Modification of Polylactic Acid (PLA) Fibers for Use as Tissue Engineering Scaffolds, ***N.M. Tambe***, *M.W. King, A. El Shafei*, North Carolina State University

Organ failure is one of the major problems faced by patients around the world. In the USA, there are currently millions of people waiting for organ transplantation with the annual healthcare costs for the treatment of these patients exceeding $500 billion. The goal of this study is to develop an innovative, resorbable porous tissue engineering (TE) scaffold with controlled biodegradability, good cytocompatibility, suitable surface chemistry for cell growth and optimum mechanical properties. Most scaffold materials do not possess any bioactivity and do not have the ability to promote extracellular matrix (ECM) secretion and support cell growth. So, the surface chemistry is very important as the outermost functional group is responsible for binding cells to the material.

In order to achieve these goals, there are a series of specific objectives:

1) To identify and compare various polymer surface modification techniques

2) To determine and study the process and material variables for those techniques

3) To characterize these techniques for changes in surface chemistry

4) To attach bioactive coatings onto the surface successfully.

Polylactic acid (PLA) was chosen as the scaffold material due to the fact that it has most of the desired TE scaffold properties. A PLA nonwoven web from Ahlstrom Nonwovens was used as the material. Surface modification was performed by thermally initiated free radical polymerization using vinyl monomers such as maleic acid and maleic anhydride. Potassium persulfate was used as the initiator and Mohr's salt was added to reduce the extent of homopolymerization. The grafted surfaces were coated with a bioactive coating of collagen using a spacer molecule called genipin. The grafted surfaces were then evaluated via Fourier transform infrared spectroscopy (FTIR), contact angle measurements, X-ray photoelectron spectroscopy (XPS), and dyeing with a basic dyestuff and using visible spectrophotometry.

The FTIR spectra show the grafting of carboxylic acid groups of maleic acid onto the surface of the PLA. The effect of monomer concentration is seen in the contact angle measurements. With increase in the monomer concentration, the contact angle fell, indicating an increase in the hydrophilicity of the material. The collagen was also successfully coated onto the grafted surface which is confirmed by the FTIR spectra showing the characteristic amide bands. Further evaluation of the surfaces is continuing with dyeing, XPS, and measuring the biological performance. In addition, other surface modification methods like plasma, atom-transfer radical polymerization (ATRP), are being undertaken.

BI-ThP3 Application of Layer-By-Layer Coatings to Tissue Scaffolds – A Novel Approach for Developing a Pro-Angiogenic Biomaterial, ***C.D. Easton***, CSIRO Materials Science and Engineering, Australia*, S.L. McArthur,* Swinburne University of Technology, Australia*, A.J. Bullock, S. MacNeil*, University of Sheffield, UK

Development of flexible coating strategies for angiogenesis promotion is critical for the health care industry to effectively treat chronic non-healing wounds. This need will continue to intensify in light of the increasing number of patients diagnosed with diabetes and an ageing population. In addition, such strategies are required within the tissue engineering community to overcome issues associated with engineered materials failing to engraft as a result of delays in neovascularisation.

An important requirement for a pro-angiogenic biomaterial is the ability to maintain a regulated release of bioactive growth factors to the wound site through the use of heparin. A number of strategies for the use of natural and synthetic heparin-mimetics have been developed, however in general they fail as the heparin can be rapidly lost from the wound site. Therefore new strategies to effectively immobilise heparin for the release of bioactive growth factors are being developed.

This work describes a layer-by-layer (LBL) approach that provides a platform for immobilising a significant amount of heparin to the substrate of choice. The LBL coating prevents desorption of immobilised heparin as a result of 'covalent bond-like' interactions from the electrostatic attraction between the multiple layers of polyelectrolytes. Employing plasma polymerisation, a thin functional coating was applied to the substrate to provide the necessary surface charge in order to build the LBL architecture. In this particular case, plasma polymerised acrylic acid (ppAAc) was deposited onto Si wafer and two different tissue scaffolds, a commercially available polypropylene type, and an electrospun PLGA (75:25) type previously reported in the literature. The LBL structure was then created by physically absorbing alternative layers of polyethyleneimine (PEI) and poly(acrylic acid) (pAAc), and once the desired number of layers was achieved, heparin was immobilised to the structures. The kinetics of the fabrication procedure was examined using a quartz crystal microbalance with dissipation (QCM-D) system, while the resulting structures were probed using X-ray Photoelectron Spectroscopy (XPS) and Atomic Force Microscopy (AFM). The effect of pH of the LBL solutions (PEI and pAAc) on the resulting structure and ability to immobilise and retain heparin were examined. This technique allowed for the immobilisation of a significant amount of heparin to the test substrates explored, more so than that previously reported using similar approaches. This approach therefore provides an inexpensive and easily expandable coating methodology for applying a pro-angiogenic interface to tissue engineered materials

BI-ThP4 In Situ Surface Initiated Enzymatic DNA Polymerization: Potential for Multiplexed Molecular Detection, ***L. Tang***, *V. Tjong, A. Chilkoti, S. Zauscher*, Duke University

We present a new technique called surface initiated enzymatic polymerization (SIEP), which uses terminal deoxynucleotidyl transferase (TdT), a template-independent DNA polymerase that catalyzes the sequential addition of deoxynucleotides (dNTPs) at the 3'-OH group of an oligonucleotide primer. We utilized TdT's ability to polymerize a long DNA chain while incorporating non-natural chemically reactive dNTPs to create new functional materials and to generate signal amplification. Previously, we have shown that fluorescent-dNTP can be directly polymerized by TdT. In this work, we will show the incorporation of amine- and aldehyde-modified dNTPs to impart reactive moieties into the polymerized DNA chain. We quantified the number of reactive dNTPs and their effect on the polymerization efficiency. We then further investigated the reactivity of the functional group for subsequent reactions, which include fluorescent dye conjugation for signal amplification, selective DNA metallization, and oligonucleotide conjugation to create a branched structure. We found that multiple reactive groups can be incorporated and they are active for subsequent reactions. For characterization of SIEP, we utilized a quartz crystal microbalance with dissipation monitoring (QCM-D) to monitor increase in surface mass during DNA polymerization in real-

time. The mass increase versus concentrations of dNTP allows us to determine the reaction constant, which reflects the growth kinetics of DNA polymerization on the surface. In addition, we examined the effect of grafting density on the polymerization reaction and the conformation of DNA brushes.

BI-ThP5 Plasma-based Approaches for Biointerface Preparation, *E.H. Lock,* ***S.H. North****, S.G. Walton, C.R. Taitt*, Naval Research Laboratory (NRL)

The ability to predict and manipulate biomolecule behavior at the biointerface determines the success of biomaterials in applications ranging from biosensing to medical devices and therapeutic products. However, precise biointerface engineering will remain elusive until the roles of physical and chemical properties of surfaces on abiotic and biotic interfacial interactions are well understood. We have shown that plasma treatment of polymers generates chemically reactive surfaces for successful silanization and biomolecule immobilization [1, 2]. The focus of this work is to investigate the influences of surface chemistry and surface morphology on biomolecule attachment. We fine-tune our plasma system to favor the production of specific functional groups that promote subsequent biomolecule attachment. The effects of surface morphology on biomolecule immobilization are also assessed. Plasma diagnostics and modeling allows us to elucidate the effects of plasma parameters (plasma density, electron temperature and the resulting kinetic ion energy) on the polymer surface modifications. The work was supported by the Office of Naval Research.

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BI-ThP6 A Novel ALD Al_2O_3-Parylene Bi-Layer Encapsulation for Biomedical Implantable Devices, ***X. Xie****, L.W. Rieth, F. Solzbacher*, University of Utah

Atomic layer deposited (ALD) Al_2O_3 has been widely used as encapsulation material for organic LEDs and solar cells due to its low water vapor transmission rate (WVTR) (~5×10^{-6} g-H_2O/m^2-day). However, its coating performance for implantable devices still needs investigation. Parylene has been commonly applied as encapsulation for implantable devices, such as Utah Electrode Arrays (UEAs). The idea of combing Al_2O_3 and parylene is based on the concept that Al_2O_3 works as moisture barrier and parylene as ion barrier. In this paper, Al_2O_3 was deposited by both thermal and plasma-enhanced ALD on interdigitated electrodes (IDEs) for comparison. AFM micrographs (Fig. 1) show that Al_2O_3 films deposited on silica substrate (RMS surface roughness of 0.17 nm) by thermal and plasma-enhanced ALD have RMS surface roughness of 0.51 nm and 0.48 nm, respectively. XPS shows that ALD films had an oxygen to aluminum ratio of 1.2 while thermal ALD Al_2O_3 is 1.09, indicating that the former is closer to Al_2O_3. A 6-μm thick parylene-C layer was deposited by CVD using Gorman process on top of Al_2O_3 and saline A-174 (Momentive Performance Materials) was used used as adhesion promoter. The samples were soaked in 1× PBS at 37 °C and 57 °C for accelerated lifetime test. Electrochemical impedance spectroscopy (EIS) and chronoamperometry were used to evaluate the performance of the encapsulation. Preliminary data shows that the leakage current (Fig. 2) remained very small (~ 7 pA) and the electrochemical impedance (Fig. 3) was consistently high (~ 3 MΩ at 1 kHz) after 5 days of soaking test at 57 °C (equivalent to at least 20 days of soaking test at 37 °C). Comparing with parylene and Al_2O_3 control samples, the Al_2O_3-parylene coated sample showed lower leakage current (Fig. 2). The impedance for three different types of samples was almost the same. However, the phase of parylene-C coated sample slightly declined after 5 days of soaking test (Fig. 3), suggesting that sample coated with Al_2O_3 had lower WVTR. No obvious difference has been observed yet for samples soaked at different temperatures since the soaking period is relatively short. Parylene came off after one day of soaking test for one sample, which might be caused by the poor adhesion between Al_2O_3 and parylene. In conclusion, preliminary results shows that the Al_2O_3-parylene bi-layer encapsulation scheme is promising encapsulation in terms of leakage current and electrochemical impedance. Long-term soaking tests are being performed to further investigate the functionality of this novel encapsulation scheme.

BI-ThP7 Complementary Electronic and Vibrational Circular Dichroism Analysis of Bovine Serum Albumin Adsorbed on Hydroxyapatite Microspheres, ***K.P. Fears****,* Naval Research Laboratory*, D.E. Day,* Missouri University of Science and Technology*, D.Y. Petrovykh,* International Iberian Nanotechnology Laboratory*, T.D. Clark*, Naval Research Laboratory

Bovine serum albumin (BSA) is a widely studied globular protein that contains ca. 68% of alpha-helices and <2% of beta-sheets in its native conformation. The well characterized secondary structure of BSA is commonly used as a benchmark for electronic (ECD) and vibrational (VCD) studies of proteins in solution. Both ECD and VCD indicated a substantial loss of helical structure accompanied by an increase of beta-sheet character in BSA thermally denatured in solution. In surface adsorption experiments, hydroxyapatite microspheres were incubated in solutions with low (1.0 mg/mL) or high (50.0 mg/mL) concentrations of BSA for one hour, then triple rinsed and re-suspended in buffer for analysis. The ECD spectra were similar for BSA adsorbed from low and high concentration solutions, both showing a sizeable increase in beta-sheet character upon adsorption, while being dominated by alpha-helical features. The VCD spectra also exhibited stronger peaks in the beta-sheet region upon adsorption of BSA on hydroxyapatite. VCD signal enhancement, however, was observed upon adsorption from the high concentration BSA solution, indicating the formation of macroscopic chiral structures. The analysis of proteins adsorbed on surfaces thus can be enhanced by taking advantage of the complementary sensitivities of ECD and VCD spectroscopies to the secondary structures of biomolecules.

BI-ThP9 Binding Affinities of *wt* and H93R PTEN to Lipid Membranes Containing PS and PI(4,5)P_2, ***S. Shenoy****,* Carnegie Mellon University*, A. Gericke,* Kent State University*, A.H. Ross,* University of Massachusetts Medical School*, M. Lösche*, Carnegie Mellon University and National Institute of Standards and Technology

PTEN is a phosphatidylinositolphosphate (PIP) phosphatase frequently mutated in human cancer [1]. By lowering PI(3,4,5)P_3 levels in the plasma membrane, it functions as an antagonist to PI3-kinase in the regulatory circuit that controls cell proliferation and survival. *wt* PTEN has only weak affinity to zwitterionic phosphatidylcholine (PC) membranes but a strong interaction with anionic lipids. Its C2 domain was shown to bind in a Ca^{2+} independent manner to phosphatidylserine (PS) and phosphatidylglycerol (PG), whereas a short N-terminal domain binds specifically to PI(4,5)P_2 [2,3]. H93R PTEN is an autism related mutant which has decreased phosphatase activity [4].

Using Surface Plasmon Resonance (SPR), we characterized the affinity of *wt* and H93R PTEN to tethered bilayer lipid membranes (tBLMs) that contain PC and PS, PC and PI(4,5)P_2, and PC, PS and PI(4,5)P_2. As compared with *wt* PTEN, we find that the H93R mutation is sufficient to cause significant increases in the protein's association with lipid membranes containing PS. PI(4,5)P_2 enhances the apparent binding constant for both proteins and leads to intriguing binding kinetics of the protein to the membrane. The binding of either protein to membranes containing both PS and PI(4,5)P_2 shows a biphasic behavior, suggesting two independent binding sites. This supports the hypothesis of non-competitive binding of the protein to PS and PI(4,5)P_2 [5]. We also performed neutron reflectivity experiments to determine the structure and orientation of PTEN bound to the membrane.

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BI-ThP10 Membrane Binding and Structure for Cytoplasmic Domain of Zeta Subunit of T Cell Receptor, ***P. Shekhar****,* Carnegie Mellon University*, F. Heinrich,* Carnegie Mellon University and National Institute of Standards and Technology*, K. Zimmerman,* University of Massachusetts Medical School*, M. Lösche,* Carnegie Mellon University and National Institute of Standards and Technology*, L.J. Stern*, University of Massachusetts Medical School

The cytoplasmic domain of the T-cell receptor zeta subunit, ζ_{cyt}, a cell surface protein complex responsible for binding peptide fragments of foreign antigens bound to major histocompatibility complex (MHC) proteins, is sufficient to couple receptor ligation to intracellular signaling cascades [1]. These domains carry immunoreceptor tyrosine-based activation motifs (ITAMs), *i.e.* signaling motifs that are phosphorylated by tyrosine kinases following receptor crosslinking. The phosphorylation of ITAMs is a first and obligatory step in signal transduction. ζ_{cyt} has been shown to be unstructured in aqueous solution and to assume a helical

conformation in the presence of anionic lipid vesicles [2,3]. Membrane binding and membrane-induced conformational changes likely plays an important role in signal transduction, but no direct structural information on these functionally important lipid-bound states was available so far. Using a synthetic membrane model, *i.e.*, fluid lipid bilayers tethered to planar solid supports [4,5], we report surface plasmon resonance (SPR) results on the binding kinetics and neutron reflectivity investigations of the association of the disordered ζ_{cyt} with membranes. We determine the extent to which the protein penetrates into the bilayer and discuss structural details of the ζ_{cyt}–lipid interaction.

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BI-ThP11 Microfluidic Bacterial Adhesion Assay to Characterize the Easy to Clean Properties of Surfaces, ***M.P. Arpa Sancet***, *C. Christophis, S. Bauer, A. Rosenhahn, M. Grunze*, Universität Heidelberg, Germany

Biofouling, the undesired growth of marine organisms on submerged surfaces, is a global problem with both economic and environmental consequences. The attachment of bacteria to surfaces is an important step in the biofouling process, and the development of ways to attenuate microbial attachment or to achieve their easy removal is desirable. We built a microfuidic system and applied it to determine the adhesion strength of bacterial biofilms. Marine bacteria, e.g. *Cobetia marina* are cultivated in this microfluidic device on the surfaces of interest. The bacterial detachment is caused by a hydrodynamic shear flow which is continuously increased and the removal is recorded via video microscopy. The adhesion strength is determined as the shear stress needed to detach 50% of the bacteria. The parameters incubation time, medium and increase of shear stress were varied in order to find the optimal conditions to carry out the biological assays. The applicability of the technique is demonstrated using self assembled monolayers with a different ability to bind water. Well hydrated surfaces lead to decreased adhesion strength while bacteria stick stronger to less hydrated surfaces. Based on these findings, a range of polysaccharide hydrogels were tested towards their potential to reduce adhesion strength.

Authors Index

Bold page numbers indicate the presenter

— A —

Abel, J.: AS+BI+NS-WeM11, 14
Afzali, A.: BI-MoA8, 4
Alam, M.: BI-TuA9, 11
Alexander, M.R.: BI-WeM3, 15
Alexandrov, T.: BI-WeM4, 15
Allen, S.: AS+BI+NS-WeM12, 14
Anderson, D.G.: BI-WeM3, 15
Ando, T.: AS+BI+NS-WeM1, **13**
Arey, B.W.: HI+AS+BI+NS-WeM11, **17**
Árnadóttir, L.: BI-MoM8, **2**
Arpa Sancet, M.P.: BI-ThP11, **24**
Ash, J.: BI-MoM4, 1
Atkinson, S.: BI-WeM3, 15

— B —

Baio, J.E.: BI-MoM4, 1
Bally, M.: BI-TuA10, **11**
Balsano, R.: AS+BI+NS-WeM11, 14
Bandeira, N.: BI-WeM4, 15
Barlow, D.E.: BI-WeM1, **14**
Bartis, E.: PS+BI-MoA10, 8; PS+BI-MoA7, **7**
Bartling, B.: PS+BI-MoA11, 8
Bauer, S.: BI-ThP11, 24
Benitez, A.S.: BI-ThM12, 20
Benítez, J.J.: BI-MoA11, 4
Bennett-Kennett, R.B.: BI-ThM12, 20
Bentley, W.E.: BN+NM-TuM11, 10; BN+NM-TuM2, 9; BN+NM-TuM3, 9; BN+NM-TuM6, 9
Betz, J.: BN+NM-TuM11, **10**; BN+NM-TuM2, 9; BN+NM-TuM6, 9
Beyer, A.: HI+AS+BI+NS-WeM6, **16**
Biffinger, J.C.: BI-WeM1, 14
Biggs, M.: BN-TuA3, 12
Blomfield, C.J.: BI-ThM4, 20
Bonnell, D.A.: AS+BI+NS-WeM10, 14; AS+BI+NS-WeM9, 14
Bradley, Q.X.: BI-ThM12, 20
Bratescu, M.A.: PS+BI-MoA4, **7**
Brueck, S.: BI+AS+NS+SS-WeA9, 19
Bruggeman, P.J.: PS+BI-MoA2, **6**
Brukman, M.J.: AS+BI+NS-WeM10, **14**
Bullock, A.J.: BI-ThP3, 22
Bunning, T.J.: AS+BI+NS-WeM6, 13

— C —

Cahen, D.: BI-MoM11, **2**
Cans, AS.: BI-MoM3, 1
Casal, P.: BI-MoA4, 3
Castner, D.G.: BI-MoM4, 1; BI-MoM6, 2
Ceccone, G.: BI-ThM6, 20
Chang, C.-Y.: BI-WeM3, 15
Chen, X.: AS+BI+NS-WeM9, **14**
Cheng, Y.: BN+NM-TuM11, 10; BN+NM-TuM2, **9**; BN+NM-TuM6, 9
Chilkoti, A.: BI-ThP4, 22
Christophis, C.: BI-ThP11, 24; BI-WeM11, 16
Chu, J.-W.: PS+BI-MoA10, 8; PS+BI-MoA7, 7
Chung, T.-Y.: PS+BI-MoA10, **8**; PS+BI-MoA7, 7
Cipriany, B.R.: BI-MoA11, 4
Clark, K.: BI-MoM9, 2
Clark, T.D.: BI-ThP7, 23
Colpo, P.: BN-TuA4, 12
Coneski, P.N.: MB+BI+PS-MoA10, **6**
Coultas, S.J.: BI-ThM4, 20
Craighead, H.G.: BI-MoA11, 4
Culbertson, R.J.: BI-ThM12, **20**

— D —

Dalby, M.J.: BI-MoM1, 1
Davies, M.C.: BI-WeM3, 15
Day, D.E.: BI-ThP7, 23
D'Emic, C.: BI-MoA8, **4**
Deshmukh, R.: MB+BI+PS-MoA1, 5
Desikan, R.P.: BI-MoA7, **4**
Dhayal, M.: BI-MoA9, 4; PS+BI-MoA8, 7
Diesner, M.-O.: BI-MoM5, 1
Discher, B.M.: AS+BI+NS-WeM9, 14
Dorrestein, P.C.: BI-WeM4, **15**
Drobny, G.P.: BI-MoM4, 1
Dubey, M.: BI-MoM4, 1

— E —

Easton, C.D.: BI-ThP3, **22**
El Shafei, A.: BI-ThP2, 22
Eliad, L.: AS+BI+NS-WeM6, 13
El-Zubir, O.: BI+AS+NS+SS-WeA9, 19
Escobar, C.A.: BI+AS+NS+SS-WeA11, **19**
Estrada-Raygoza, I.C.: PS+BI-MoA6, **7**

— F —

Fazio, T.: BN-TuA3, 12
Fears, K.P.: BI-ThP7, **23**
Figueroa, J.: BI-MoA10, **4**
Fischer, D.A.: BI-MoM4, 1
Fletcher, B.: BI-MoA8, 4
Fujita, D.: HI+AS+BI+NS-WeM12, 17

— G —

Gamble, L.J.: BI-MoM8, 2; BI-WeM6, 15
Garramone, J.J.: AS+BI+NS-WeM11, **14**
Gazit, E.: AS+BI+NS-WeM6, 13
Gericke, A.: BI-ThP9, 23
Goeckner, M.J.: PS+BI-MoA6, 7
Gölzhäuser, A.: HI+AS+BI+NS-WeM6, 16
Gomez, D.: BI-MoA10, 4
Gonzalez, D.: BI-WeM4, 15
Goodson, W.J.: BI-WeM1, 14
Gordonov, T.: BN+NM-TuM3, **9**
Graves, D.B.: PS+BI-MoA10, 8; PS+BI-MoA7, 7
Grunze, M.: BI-ThP11, 24; BI-WeM11, 16
Guillorn, M.A.: BI-MoA8, 4
Gunnarsson, A.: BI-MoM3, 1
Guo, H.X.: HI+AS+BI+NS-WeM12, **17**

— H —

Hamby, M.: BI-WeM4, 15
Hamers, R.J.: BI+AS+NS+SS-WeA3, 18
Han, S.M.: BI-MoA3, 3
Hanuš, J.: BI-ThM6, 20
Hardy, G.: BI-TuA9, **11**
Harl, R.R.: BI+AS+NS+SS-WeA10, 19
Hart, M.A.: BI-ThM12, 20
Hayashi, N.: PS+BI-MoA9, 8
Heinrich, F.: BI-ThP10, 23
Henneken, C.: BI-MoM9, 2
Herbots, N.X.: BI-ThM12, 20
Hillenmeyer, E.: BI-MoM6, **2**
Hla, S.-W.: BI-MoM9, 2
Hlawacek, G.: HI+AS+BI+NS-WeM5, 16
Ho, A.D.: BI-WeM11, 16
Hockenberry, D.: BI-WeM6, 15
Holbrook, R.D.: BI+AS+NS+SS-WeA3, 18
Hook, A.L.: BI-WeM3, **15**
Höök, F.: BI-MoM3, 1; BI-TuA10, 11
Hung, C.H.: MB+BI+PS-MoA11, 6
Hutton, S.J.: BI-ThM4, **20**

— I —

Iijima, T.: HI+AS+BI+NS-WeM3, 16
Itoh, H.: HI+AS+BI+NS-WeM12, 17
Ivory, C.F.: BI-MoA3, 3
Iwasaki, T.: HI+AS+BI+NS-WeM12, 17
Iwaya, T.: AS+BI+NS-WeM5, 13

— J —

Jayawarna, V.: BI-MoM1, 1
Jaye, C.: BI-MoM4, 1
Jennings, G.K.: BI+AS+NS+SS-WeA11, 19
Johnston, E.: BI-ThM9, **20**
Jönsson, P.: BI-MoM3, 1

— K —

Kathan-Galipeau, K.: AS+BI+NS-WeM9, 14
Kawai, M.: AS+BI+NS-WeM5, 13
Kersten, R.: BI-WeM4, 15
Khademhosseini, A.: BN-TuA1, **12**
Khaing, A.: BN+NM-TuM5, **9**
Khan, S.: BI-MoM9, **2**
Kim, H.: BI-MoA4, 3
King, M.W.: BI-ThP2, 22
Kitazaki, S.: PS+BI-MoA9, **8**
Kobayashi, M.: MB+BI+PS-MoA8, **5**
Koelsch, P.: BI-MoM5, **1**
Koga, K.: PS+BI-MoA9, 8
Korolkov, V.V.: AS+BI+NS-WeM12, **14**
Kummel, A.C.: BI-ThM11, 20
Kurczy, M.: BI-MoM3, 1
Kutz, T.: BI-ThM12, 20
Kwong, H.M.: BI-ThM12, 20

— L —

LaBean, T.: BI-MoM10, 2
LaBella, V.P.: AS+BI+NS-WeM11, 14
Laibinis, P.: BI+AS+NS+SS-WeA10, 19
Lambert, C.: BN+NM-TuM5, 9
Lamsa, A.: BI-WeM4, 15
Langer, R.: BI-WeM3, 15
Langhammer, C.B.: BI+AS+NS+SS-WeA4, 18
Larson, G.: BI-TuA10, 11
Larsson, E.M.K.: BI+AS+NS+SS-WeA4, 18
Latulippe, D.R.: BI-MoA11, 4
Lawrie, J.: BI+AS+NS+SS-WeA10, **19**
Lee, S.: BI-MoA4, 3
Lee, S.W.: PS+BI-MoA1, **6**; PS+BI-MoA11, 8
Leggett, G.: BI+AS+NS+SS-WeA9, **19**
Li, K.: BI-MoM4, 1
Lidzey, D.: BI+AS+NS+SS-WeA9, 19
Lim, D.V.: BI-MoA10, 4
Liu, C.-C.: PS+BI-MoA11, 8
Liu, W.-T.: BI-WeM4, 15
Lock, E.H.: BI-ThP5, 23
Lopez, G.P.: BI+AS+NS+SS-WeA9, 19
Lösche, M.: BI-ThP10, 23; BI-ThP9, 23; BI-TuA7, **11**
Louis, K.: BI+AS+NS+SS-WeA3, 18
Lu, W.: BI-MoA4, 3; BI-MoA6, 3
Luo, X.L.: BN+NM-TuM2, 9; BN+NM-TuM6, **9**

— M —

MacNeil, S.: BI-ThP3, 22
Magana, S.: BI-MoA10, 4
Marchi, A.: BI-MoM10, 2
Martin, T.: BN-TuA4, 12
Matsunaga, S.: AS+BI+NS-WeM5, 13
Maziarz, A.: BN+NM-TuM5, 9
McArthur, S.L.: BI-MoM1, **1**; BI-ThP3, 22
McCarthy, J.: BI+AS+NS+SS-WeA3, 18
McDevitt, T.: BI-WeM9, **15**
McGimpsey, W.: BN+NM-TuM5, 9
Mehn, D.: BN-TuA4, 12
Mendez, N.: BI-ThM11, **20**
Meseck, G.R.: BI-WeM11, 16
Messmer, B.T.: BI-ThM11, 20
Milkani, E.: BN+NM-TuM5, 9
Moffitt, C.E.: BI-ThM4, 20
Moore, B.: BI-WeM4, 15
Morrish, F.: BI-WeM6, 15
Murphy, A.M.: BI-ThM12, 20
Murphy, P.J.: BI-MoA11, 4

— N —

Naik, R.R.: AS+BI+NS-WeM6, 13
Nanayakkara, S.: AS+BI+NS-WeM10, 14
Ning, N.: PS+BI-MoA10, 8; PS+BI-MoA7, 7
Ning, T.: BI-MoA8, 4
North, S.H.: BI-ThP5, **23**
Nowak, J.: BN-TuA4, **12**

— O —

Oehrlein, G.S.: PS+BI-MoA10, 8; PS+BI-MoA7, 7

Ogawa, S.: HI+AS+BI+NS-WeM3, **16**
Onishi, K.: HI+AS+BI+NS-WeM12, 17
Orr, G.: HI+AS+BI+NS-WeM11, 17
Overzet, L.J.: PS+BI-MoA6, 7

— P —

Padron-Wells, G.: PS+BI-MoA6, 7
Page, S.C.: BI-ThM4, 20
Palma, M.: BN-TuA3, 12
Pandey, R.R.: BI-MoA9, **4**; PS+BI-MoA8, 7
Park, D.-G.: BI-MoA8, 4
Payne, G.F.: BN+NM-TuM11, 10; BN+NM-TuM2, 9
Pecht, I.: BI-MoM11, 2
Pehrsson, P.E.: BI-WeM1, 14
Penkala, R.: BI-MoM6, 2
Petersen, E.R.: BI-WeM1, 14
Petrovykh, D.Y.: BI-ThP7, 23
Pickard, D.S.: HI+AS+BI+NS-WeM9, **16**
Piqueras Jover, R.: BN-TuA3, 12
Poelsema, B.: HI+AS+BI+NS-WeM5, 16
Pogliano, K.: BI-WeM4, 15

— R —

Rauh, E.: BI-MoM9, 2
Reed, S.M.: BI-TuA11, 11
Remmert, J.L.: AS+BI+NS-WeM6, **13**
Rettenmayr, M.: BI-WeM2, 14
Richmonds, C.: PS+BI-MoA11, 8
Rieth, L.W.: BI-ThP6, 23
Roberts, A.J.: BI-ThM4, 20
Roberts, C.J.: AS+BI+NS-WeM12, 14
Robinson, M.: BI-WeM6, **15**
Rogers, B.R.: BI+AS+NS+SS-WeA10, 19
Rosenhahn, A.: BI-ThP11, 24; BI-WeM11, **16**
Ross, A.H.: BI-ThP9, 23
Rossi, F.J.: BI-ThM6, **20**; BN-TuA4, 12
Roy, S.: BI-MoM1, 1
Rubloff, G.W.: BN+NM-TuM11, 10; BN+NM-TuM2, 9; BN+NM-TuM6, 9
Ruidiaz, M.E.: BI-ThM11, 20
Russell, J.N.: BI-WeM1, 14

— S —

Saaem, I.: BI-MoM10, **2**
Saini, K.K.: BI-MoA9, 4; PS+BI-MoA8, **7**
Saito, N.: PS+BI-MoA4, 7
Sakurai, S.: BI-ThP1, **22**
Salvati, L.: BI-ThM2, **20**
Sanchez, A.B.: BI-ThM11, 20
Sankaran, R.M.: PS+BI-MoA1, 6; PS+BI-MoA11, 8
Schetz, J.A.: MB+BI+PS-MoA1, **5**
Schlaf, R.: BI-MoA10, 4
Schönherr, H.: BI+AS+NS+SS-WeA7, **18**
Schroeter, A.: BI-WeM2, 14
Schuster, S.: BI-WeM2, 14
Schvartzman, M.: BN-TuA3, 12
Schweikert, E.A.: BI+AS+NS+SS-WeA1, **18**
Sell, C.H.: BI-ThM12, 20
Sell, D.A.: BI-ThM12, 20
Seog, J.: PS+BI-MoA10, 8; PS+BI-MoA7, 7
Sepunaru, L.: BI-MoM11, 2
Serrano, A.: MB+BI+PS-MoA4, **5**
Sheetz, M.: BN-TuA3, 12
Shekhar, P.: BI-ThP10, **23**
Shenoy, S.: BI-ThP9, **23**
Sheth, P.: MB+BI+PS-MoA1, 5
Sheves, M.: BI-MoM11, 2
Shiratani, M.: PS+BI-MoA9, 8
Shuler, M.L.: BN+NM-TuM9, **9**
Shumaker-Perry, J.S.: BI-MoA1, **3**
Shutthanandan, V.: HI+AS+BI+NS-WeM11, 17
Siegismund, D.: BI-WeM2, **14**
Simonsson, L.: BI-MoM3, **1**
Soloway, P.D.: BI-MoA11, 4
Solzbacher, F.: BI-ThP6, 23
Spencer, N.D.: MB+BI+PS-MoA4, 5; MB+BI+PS-MoA9, 6
Stern, L.J.: BI-ThP10, 23
Sterner, O.: MB+BI+PS-MoA9, **6**
Sun, J.C.: MB+BI+PS-MoA11, 6
Surman, D.J.: BI-ThM4, 20
Szakal, C.: BI+AS+NS+SS-WeA3, **18**

— T —

Taitt, C.R.: BI-ThP5, 23
Takahara, A.: BI-ThP1, 22; MB+BI+PS-MoA8, 5
Takai, O.: PS+BI-MoA4, 7
Tambe, N.M.: BI-ThP2, **22**
Tang, L.: BI-ThP4, **22**
Taubert, I.: BI-WeM11, 16
Tendler, S.J.B.: AS+BI+NS-WeM12, 14
Terada, M.: MB+BI+PS-MoA8, 5
Thamban, P.L.S.: PS+BI-MoA6, 7
Thomas, W.: BI-MoM6, 2
Thundat, T.G.: BI-MoA7, 4
Tian, H.C.: BI-MoA11, 4
Tian, J.: BI-MoM10, 2
Timmons, R.B.: MB+BI+PS-MoA1, 5
Tizazu, G.: BI+AS+NS+SS-WeA9, 19
Tjong, V.: BI-ThP4, 22
Tolic, A.: HI+AS+BI+NS-WeM11, 17
Topolancik, J.: BI-MoA11, **4**
Tosatti, S.: MB+BI+PS-MoA4, 5; MB+BI+PS-MoA9, 6
Tribby, L.: BI-MoA3, **3**
Tsao, C.Y.: BN+NM-TuM11, 10; BN+NM-TuM2, 9; BN+NM-TuM6, 9
Turchanin, A.: HI+AS+BI+NS-WeM6, 16

— U —

Uljin, R.V.: BI-MoM1, 1

— V —

van Gastel, R.: HI+AS+BI+NS-WeM5, 16
Van Neste, C.W.: BI-MoA7, 4
Van Swol, F.: BI-MoA3, 3
Vass, S.: BI-ThM2, 20
Vasudev, M.C.: AS+BI+NS-WeM6, 13
Veligura, V.: HI+AS+BI+NS-WeM5, **16**
Voevodin, A.A.: AS+BI+NS-WeM6, 13

— W —

Wainright, J.: PS+BI-MoA11, 8
Wallin, C.B.: BI-MoA11, 4
Walton, S.G.: BI-ThP5, 23
Wang, M.S.: BI-TuA11, **11**
Wang, Y.: BI-MoA6, **3**
Watanabe, H.: BI-ThP1, 22
Watrous, J.: BI-WeM4, 15
Webster, D.C.: MB+BI+PS-MoA6, **5**
Weidner, T.: BI-MoM4, **1**
Weise, N.K.: MB+BI+PS-MoA10, 6
Weiss, S.M.: BI+AS+NS+SS-WeA10, 19
Wen, X.: BI-MoA4, **3**
Wilkens, B.J.: BI-ThM12, 20
Williams, P.: BI-WeM3, 15
Wind, S.: BN-TuA3, **12**
Witzke, M.: PS+BI-MoA11, **8**
Wu, H.C.: BN+NM-TuM2, 9; BN+NM-TuM6, 9
Wu, J.K.: MB+BI+PS-MoA11, 6
Wu, K.R.: MB+BI+PS-MoA11, **6**
Wynne, J.H.: MB+BI+PS-MoA10, 6

— X —

Xie, X.: BI-ThP6, **23**
Xie, Y.: HI+AS+BI+NS-WeM11, 17

— Y —

Yakovenko, O.: BI-MoM6, 2
Yamada, T.: AS+BI+NS-WeM5, **13**
Yang, J.: BI-WeM3, 15
Yeh, C.W.: MB+BI+PS-MoA11, 6
Yip, C.: AS+BI+NS-WeM3, **13**

— Z —

Zafar, S.: BI-MoA8, 4
Zandvliet, H.: HI+AS+BI+NS-WeM5, 16
Zauscher, S.: BI-ThP4, 22; BI-TuA9, 11
Zhdanov, V.P.: BI+AS+NS+SS-WeA4, 18
Zimmerman, K.: BI-ThP10, 23
Zoric, I.L.: BI+AS+NS+SS-WeA4, **18**
Zulkifli, A.R.: BI+AS+NS+SS-WeA11, 19
Zürcher, S.: MB+BI+PS-MoA4, 5; MB+BI+PS-MoA9, 6
Zurn, M.: BN-TuA4, 12