Monday Morning, November 9, 2009

Biomaterial Interfaces
Room: K - Session BI+NS-MoM

Nanoparticles and Self Assembly
Moderator: A. Chilkoti, Duke University, V. Perez-Luna, Illinois Institute of Technology

8:20am **BI+NS-MoM1 Self-assembling and Biofunctionalization of Plasmonic Gallium and Gold Nanoparticles on Semiconductors for Label Free Bio- and Chemo-sensing**, *M. Losurdo,* IMIP-CNR, Italy*, P.C. Wu,* Duke University*, G.V. Bianco, M. Giangregorio,* IMIP-CNR, Italy*, T.H. Kim,* Duke University*, G. Bruno,* IMIP-CNR, Italy*, **A.S. Brown***, Duke University

Biorganic functionalization of gold thin films and nanocluster mesoscalar assemblies and their resulting optical properties have immense applications ranging from biosensing to nanomedicine. The appealing property is the surface plasmon resonance of those bio-metal ensembles that can be tailored not only by the metal nanoparticles geometry but also by the nature of the biomolecules and the chemistry of the interface. This characteristic is being exploited for realizing nanoscale optical chemosensors and biosensors based on localized surface plasmon resonance of metal nanoparticles.

In this contribution we present and discuss bio- and chemo-functionalization of a novel system based on plasmonic gallium (Ga) nanoparticles (NPs) self-assembled on a large variety of semiconductor substrates including Si, SiC, GaN and GaAs. The advantages of this system for a plasmonic metal/semiconductor sensing platform are presented and compared to the standard gold (Au) nanoparticles system.

The Ga and Au NPs are directly deposited on semiconductors by evaporation and/or plasma sputtering.

For both systems Ga (NPs)/semiconductor and Au(NPs)/semiconductor we discuss semiconductor surface treatments affecting the interface chemistry and the dynamics of interface phenomena playing a role in tailoring the surface plasmon resonance.

Those systems have also been functionalized by bio-molecules, e.g., antigens and antibodies for biosensing and by porphyrins for gas sensing to nitric oxide (NO).

The peculiarity of our study is the exploitation of Plasmonic spectroscopic ellipsometry (HORIBA UVISEL, Jobin Yvon) for the optical monitoring of the plasmon resonance tuning in real time during the nanoparticles deposition on semiconductor surfaces and for the functionalization of metal nanoparticles by anchoring biomolecules to the semiconductor nanostructures.

For these plasmon-enhanced semiconductor nanoscale sensors, the degree of coverage by the porphyrin and/or protein, the aggregation, the type of binding and orientation of biomolecules on a semiconductor nanostructures surface is important for the sensing activity. Therefore, plasmonic ellipsometry is used to investigate the interplay among the nanostructures size and shape, the functionalizing solution concentration and dipping time, the mechanism of anchoring of the functionalizing molecules.

Data are corroborated by atomic force and electrical force microscopies.

This work is supported by the 7FP European Project NanoCharM_Multifunctional NanoMaterial Characterization exploiting ellipsometry and polarimetry

8:40am **BI+NS-MoM2 Geometry and Interactions: How Shape and Intermolecular Interactions Direct the Self-Assembly of Cage Molecules on Au{111}**, ***J.N. Hohman***, *M. Kim, P. Zhang, E.I. Morin,* The Pennsylvania State University*, V.E. Balema,* The Sigma Aldrich Corporation*, P.S. Weiss*, The Pennsylvania State University

The self-assembly of cage molecules on metal surfaces enables the manipulation of interface chemistry while eliminating an entire class of defect modes commonly associated with straight-chain molecules such as the n-alkanethiols. The adamantanethiols and carboranethiols have similar geometries and unit cells on Au{111}, but exhibit striking differences in their behavior. We attribute these differences to their contrasting electronic structures and consequent intermolecular interactions. We have characterized the SAMs of positional isomers of several carboranethiols and adamantanethiols by scanning tunneling microscopy, cyclic voltammetry, grazing-incidence Fourier transform infrared spectroscopy, Kelvin probe microscopy, and contact-angle goniometry. We discuss how geometry and intermolecular interactions play competing roles in determining monolayer assembly and stability.

9:00am **BI+NS-MoM3 On the Role of Supramolecular Nanostructure in determining Interfacial Energy and Biological Interactions**, ***F. Stellacci***, Massachusetts Institute of Technology **INVITED**

It is know that specific molecules can spontaneously arrange on various surfaces forming two-dimensional poly-crystalline mono-molecular layers called self-assembled monolayers (SAMs). These organic coatings are used to impart targeted optical, electronic and biological properties to surfaces. Very often SAMs composed of more than one type of molecule (mixed-SAMs) are used to simultaneously impart multiple properties. Scanning tunneling microscopy (STM) studies have shown that, in mixed SAMs, molecules phase-separate in domains of random shape and size.

We will show that when mixed SAMs are formed on surfaces with a radius of curvature smaller than 20 nm they spontaneously phase-separate in highly ordered phases of unprecedented size. The reason for this supramolecular phenomenon is purely topological and can be rationalized through the “hairy ball theorem”. In the specific case of mixed SAMs formed on the surface of gold nanoparticles, the molecular ligands separate into 5 Å wide phases of alternating composition that encircle or spiral around the particle metallic core. This new family of nano-structured nano-materials shows new properties solely due to this novel and unique morphology. For example, we will show that the cell uptake of these particles strongly depends on the particle’s composition and the ligand shell morphology.

9:40am **BI+NS-MoM5 Can We Make Alkanethiol SAM's on Surface of Gold Nanorods?**, ***S. Chakraborty****, S. Lee, V. Perez-Luna*, Illinois Institute of Technology

Gold has been a fascinating element that drew the interest of people since early origins of human history. However, it is just over the last two decades that it gained a resurgence of interest, in conjunction with the advent and development of nanotechnology. Gold nanoparticles have a wide variety of bio-applications such as labeling, sensing, and targeted drug delivery. A directed assembly of gold nanoparticles in the form of nanowires or networks is extensively used as nanodevices. Nanoparticles come in a variety of shapes; spheres, rods and cubes being the frequently observed ones. Surface functionalization facilitates tailoring of the gold surface for various applications. The objective here is to investigate the surface modification of gold nanorods by chemisorption of alkanethiols.

An important property of gold nanorods is their *aspect ratio,* which is defined as the width-height ratio. The lateral surface and edge surface mostly comprise of [100[and [111[surfaces respectively. By appropriate choice of capping agents, the anisotropic surfaces can be tailored for different applications. One of the most-successful methods for synthesis of gold nanorods relies on the use of cationic surfactant CetylTrimethyl Ammonium Bromide (CTAB). CTAB provides stability, size and shape control. The displacement of CTAB allows fuctionalization with other molecules. In this work, the displacement of CTAB is carried out with alkanethiols of varying chain length, viz. 16-mercaptohexadecanoic acid, 11- mercaptoundecanoic acid, 3-mercapto propionic acid. An indirect investigation method is used to analyze the behavior of gold nanorods. Planar surfaces with the same crystalline structures as gold nanorods (111 and 110) are selected for the experiment. Surface modification of planar surfaces is conducted in two steps: formation of CTAB bilayers by immersing planar substrates followed by displacement of CTAB bilayers with alkanethiols.

The experimental studies are characterized by contact angle measurements, fourier transform infrared spectroscopy and cyclic voltammetry. The displacement of CTAB is confirmed by decrease in the contact angle upon formation of carboxyl terminated surfaces and concomitant appearance of stretching band of carboxyl groups. Cyclic voltammetry studies are carried out to calculate the free energy of adsorption of the alkanethiol layers on gold surfaces. Results indicate that longer chain alkanethiols perform better in terms of displacing the CTAB layer from the gold surfaces. The displacement of CTAB is possible on both the lateral and edge surfaces.

10:00am **BI+NS-MoM6 Size-selective Placement of Nanoparticles on a Single Particle Level**, ***P. Bhadrachalam****, S.J. Koh*, University of Texas at Arlington

We present a new nanoparticle placement technique in which single nanoparticles of different sizes recognize different target positions on a substrate and exactly one nanoparticle of specific size is placed on each target position in a self-limiting way. We demonstrate this by using ~50nm and ~20nm colloidal Au nanoparticles (AuNPs) as a model system, where the ~50nm particles are first electrostatically guided onto targeted substrate locations and then the ~20nm particles to different target locations on the

same substrate. The electrostatic guiding structure was defined using CMOS-compatible fabrication processes and subsequent functionalization of surfaces using self-assembled monolayers (SAMs) of organic molecules. Using appropriate guiding structure, we present >90% success rate of ~50nm AuNPs placement onto substrate locations targeted for ~50nm AuNPs only. Theoretical calculations for ~20nm AuNPs, which was carried out by solving the non-linear Poisson-Boltzmann equation, revealed that the self-limiting single-particle placement is due to an increase of the free energy barrier after the placement of one nanoparticle onto a targeted substrate location, which prevents the approach of other nanoparticles to the already occupied position. The size-selective placement of single nanoparticles can be explained by dependence of the free energy barrier changes upon the sizes of nanoparticles and guiding structures. The same approach may also be useful for size-selective and single-entity-level placement of other nanoscale building blocks such as nanowires, proteins, and DNA.(Supported by NSF CAREER (ECS-0449958), ONR (N00014-05-1-0030), and THECB (003656-0014-2006))

Nanometer-scale Science and Technology
Room: L - Session NS+BI-MoM

Nanowires and Nanoparticles I
Moderator: L.E. Ocola, Argonne National Laboratory

8:20am **NS+BI-MoM1 Zirconia Nanoplatelets for use in Dental Restoratives**, ***K. Coulter***, *V.Z. Poenitzsch, S. Wellinghoff, B. Furman, M. Rubal, D. Nicolella, K. Chan*, Southwest Research Institute

Photopolymerizable composites containing very high volume fractions (>50%) of radio-opaque nanoplatelets in a low polymerization shrinkage, liquid crystalline (LC) monomer mixture are being developed as dental restoratives. The LC monomer promotes the organization of the nanoplatelets into either a smectic or columnar, colloidal liquid crystal structure with significantly enhanced strength. Further, theoretical calculations suggest that the tetragonal form of the yttria-stabilized zirconia nanoplatelets that SwRI is producing by a roll-to-roll, e-beam evaporation process can be induced to transform into a less dense monoclinic form in the presence of the hydrostatic tensile stresses expected at a growing crack tip within the composite resin. This transformational volume expansion coupled with the inherent ductility of the surrounding LC polymer matrix results in substantial improvements in dental composite wear resistance. SwRI has successfully coated onto a 200nm pitch, nanoembossed PET film in single run lengths of hundreds of meters, a 10 nm alkali halide release layer to produce a 50nm thick zirconia film of tetragonal crystal form which is subsequently removed by dissolving the alkali halide layer in water. The nanoembossed pattern transfers to the zirconia layer to produce nanoplatelets. three specific problems have been encountered including: (1) producing tetragonal zirconia of consistent morphology in different ca 100 meter runs, (2) complete fracture along the nanoembossed pattern, (3) color in the zirconia, most probably resulting from oxygen depletion and sub-4+ zirconia oxidation states. In this paper we will present the fabrication process and challenges in producing the nanoplatelets and present some of the preliminary composite data.

8:40am **NS+BI-MoM2 Formation of Metallic Nanoclusters on TiO_2 by Deposition and Sputtering**, ***A.B. Arjad***, *J.A. Yarmoff*, University of California, Riverside

The discovery that Au nanoclusters are catalytically active when supported in an oxide matrix[1] has led to an abundance of surface studies, particularly those concentrating on metal nanoclusters deposited on single crystal TiO_2. For example, STM showed that Au clusters grow on TiO_2(110) without a wetting layer and that the size of the clusters increases as more metal is deposited.[2] We previously used low energy alkali ion scattering to interrogate such clusters grown by evaporation, measuring the neutral fraction of the backscattered ions with time-of-flight spectroscopy.[3] We were able to show that the neutralization depends on the electronic structure of the clusters, and therefore changes as a function of cluster size. Our group also demonstrated a novel method for the production of nanoclusters by Ar^+ sputtering a thin Au film deposited on TiO_2.[4] As this initial work was performed with Au, it is not clear whether nanoclusters displaying quantum size effects could be fabricated by sputtering other materials. In the work presented here, Ag and Pt are evaporated onto TiO_2(110) and thin films of these metals are sputtered. The materials are then interrogated via the scattering of 2.5 keV Na^+. It was found that while both Pt and Ag form nanoclusters by evaporation, only Pt forms nanoclusters after sputtering. This may be due to differences in the surface free energy of the metals, or of the propensity of Ag to oxidize. Future efforts will include temperature programmed desorption to test the chemical activity of the metal nanoclusters, as well as the use of other substrates.

[1]M. Haruta, et al., J. Catal. **144**, 175 (1993).

[2]M. Valden, X. Lai and D.W. Goodman, Science **281**, 1647 (1998).

[3]G.F. Liu, Z. Sroubek and J.A. Yarmoff, Phys. Rev. Lett. **92**, 216801 (2004).

[4]P. Karmakar, G.F. Liu, Z. Sroubek and J.A. Yarmoff, Phys. Rev. Lett. **98**, 215502 (2007).

9:00am **NS+BI-MoM3 Towards Emergence of New Function by Engineering Materials: Colloidal Nanocrystals as Building Blocks for Nanocomposites and Assemblies**, ***M.L. Curri***, CNR IPCF, Italy **INVITED**

Increasing attention has been devoted to the properties of materials in nanophase state, which are highly dependent on their size and shape and can be dramatically different from those of the corresponding bulk phase materials. Such geometry dependent characteristics result essential for design novel functional materials with tailored characteristics, revealing a high impact on fundamental aspects as well as on potential technology applications. Fine tuning of the nanomaterial electronic, optical, magnetic, thermal, mechanical, and chemical properties opens possibilities for their wide applications in several technological fields. In this sense the basic nanosized building blocks of such complex structures play a crucial role and the extent to which nanocrystals (NCs) and nanoparticles (NPs) can be fabricated, engineered and their properties tailored is central. Chemical strategies are now able to provide highly processable NCs, possessing an adjustable interface with the external environment, able to tune their specific chemical reactivity towards the surroundings1. Equally decisive is the scheme used to convey the instruction for assembly and organization into the inorganic NPs. Assembly can be spontaneous (self assembly), guided by the intrinsic information coded into the building blocks, and/or driven by external stimuli, templates, chemical and/or physical forces.1,2 The complexity of the formed structures depend on a variety of factors, ranging from the type of the single structural component, the length scale along which the structured material is achieved, the kind of mechanism ultimately involved for the creation of the final architectures. The NC based structures, either hierarchical organized or nanocomposite in nature, can show original emergent properties which cannot be readily envisioned from the building block original properties, while can be observed to arise in the originated mesostructure of nano-objects.3 The potential to design and fabricate such nanostructured materials with unprecedented functionality open the routes for applications in biomedical, optical, electronic, catalytic and light energy conversion systems.

1. E. Fanizza, P. D. Cozzoli, M. L. Curri, M. Striccoli, E. Sardella, A. Agostiano (2007) Adv.Funct. Mater. 17 201-211.

2. M. L. Curri, R. Comparelli, N. Depalo, E. Fanizza, M. Striccoli "Patterning and Assembling of Inorganic Nanocrystals" in Nanomaterials: Inorganic And Bioinorganic Perspectives Edited by C. M. Lukehart and R. A. Scott, Chichester, UK: John Wiley & Sons, Ltd, pp 327-356,

3. J. Y.Kim, C. Ingrosso, V. Fakhfouri, M. Striccoli, A. Agostiano, M. L. Curri, J. Brugger (2009) Small 5 1051-1057.

9:40am **NS+BI-MoM5 The Direct Writing of Nanoscale Patterns of Nanoparticles and Polymer–Nanoparticle Composites**, ***P. Sheehan***, *W.-K. Lee,* Naval Research Laboratory, *W. King, Z. Dai*, University of Illinois, Urbana-Champaign

In thermal Dip Pen Nanolithography (tDPN), a heatable AFM cantilever regulates the deposition of an ink through controlled melting, much like a nanoscale soldering iron. Control over writing is exceptional—deposition may be turned on or off and the deposition rate easily changed without breaking surface contact. Moreover, the technique may be performed in UHV and is compatible with standard CMOS processing. tDPN has been successful at depositing materials ranging from semiconductors to insulators to metals at speeds up to 200 µm/s.

Recently, we have become interested in directly depositing nanoparticles-polymer composites. Nanoparticles and nanoparticles–polymer composites offer many new capabilities that could greatly advance nanoelectronics, data storage, biosensors, and optical imaging applications. However, these applications often require that the nanoparticles or composites be formed into nanostructures that are precisely deposited on a surface or in a device. This requirement has spurred the development of many new nanolithographies but, to date, they have exhibited relatively low resolution (>~100 nm), a lack of generality to a range of materials, or the requirement of many serial processing steps. With tDPN, we can deposit with nanoscale precision a wide range of polymers (PMMA, P(VDF-TrFE), polyethylene) that contain metallic nanoparticles, semiconducting nanoparticles, or small molecules. An oxygen plasma can remove the polymer to reveal evenly dispersed nanoparticles or, for some combinations, precisely-placed 10 nm

wide rows of nanoparticles. The flexibility and precision of this approach should greatly speed the advent of AFM tip based nanomanufacturing.

10:00am **NS+BI-MoM6 Synthesis and Structural Characterization of Fe-O Nanonetworks**, ***G.C. Franco***, *C.V. Ramana*, University of Texas at El Paso

Iron (Fe) oxides and hydroxides have been the subject of numerous investigations for many years. Fe oxides and hydroxides find application in chemical catalysis, magneto-electronics, energy storage and conversion, and nuclear industry. The properties and phenomena of these materials are influenced by the morphology and size. The present work is performed to grow high-quality and ordered structure Fe oxides by simple wet-chemical method at a relatively low temperature. Fe oxides and hydroxides have been prepared in aqueous media by precipitation of iron nitrate and ammonium hydroxide. Their growth and structural characteristics have been investigated using x-ray diffraction (XRD) and high-resolution scanning electron microscopy (HRSEM). The approach is preparing different solutions of Fe particles in suspension and acetic acid at different concentrations and subject to heat treatment at 100 °C. XRD analyses indicate that the solids obtained from aqueous stage exhibit goethite (α-FeO(OH)) with average particle size 2-3 nm, while the samples with acetic acid addition shown hematite (α-Fe_2O_3) with average size ~20 nm. HRSEM images confirm the presence of particles ~100 nm in samples with no acetic acid. HRSEM indicate that the samples with acetic acid exhibit nanoparticles ~20 nm with small spots within the particles. In addition, SEM shows the presence of net-shaped structures and particles with high-dense porosity within the nano-regime depending on the concentration of acetic acid. The experiments also confirm that Fe oxide nano-particles and networks are stable to a temperature of 500 °C. The results obtained on the growth, structure, and properties of the grown Fe-based nanomaterials will be presented and discussed in detail.

10:40am **NS+BI-MoM8 Synthesis of Shape Controlled Sinter Resistant Nanoparticles**, ***N. Shukla***, *M. Nigra, A.J. Gellman*, Carnegie Mellon University

We report synthesis of shape controlled, sinter resistant, core shell nanoparticles. The synthesis conditions allow control of the shell shape and the preparation of hexagonal shells and spherical shells. The 3D structures of these particles have been studied using angle resolved high resolution TEM. 2D cross sectional TEM images show that the cores are not positioned at the centers of the shells. By rotating the nanoparticles and monitoring the apparent motions of the cores in the 2D cross sectional images it is possible to determine the radial position of the core with respect to the center of the shell. The distribution of core positions within the core shell structures is bimodal. These observations suggest that the Fe shells grow on the Pt cores by a nucleation process rather than layer-layer growth.

11:00am **NS+BI-MoM9 Fabrication and Characterization of Carbon Nanostructures; Optical, Mechanical and Chemical Properties**, ***H. Fredriksson***, *T. Pakizeh,* Chalmers University of Technology, Sweden*, J. Cardenas,* University of Oslo, Norway*, M. Käll, B.H. Kasemo, D. Chakarov*, Chalmers University of Technology, Sweden

Nanosized carbon particles are present in many different context and applications. Examples include, aerosols and interstellar dust, inks, lubricants, composite materials and electronics. Investigations of the physical properties of such nanocarbons are therefore well motivated. We present a systematic investigation of the optical, mechanical and chemical properties of graphite and amorphous carbon nanostructures with different size. Hole-mask colloidal lithography and oxygen reactive ion etching is used to fabricate supported carbon nanostructures with well-defined diameters ranging from ~100 to 350 nm and heights from ~50 to 200 nm. Optical absorption/extinction spectra of these samples, as well as finite difference time domain (FDTD) calculations, reveal resonant absorption of visible light, both for the graphitic and for the amorphous carbon structures. The spectral maxima of the absorption peaks are correlated to the nanostructure diameters and heights. These optically resonant nanostructures are then further investigated using Raman spectroscopy, and compared with measurements from macroscopic graphite crystals. We show that the limited size has little influence on the mechanical properties of the graphite, i.e. the nanostructures have the mechanical properties of crystalline graphite. However, a slight enhancement of the Raman scattering intensity, correlated to the resonant absorption in the nanostructures is observed. Upon increasing the laser power we also observe distinct peak-splits and -shifts as well as increasing anti-Stokes signal intensity, suggesting selective heating of the nanostructures. Furthermore, the correlation between size and optical absorption have been used to follow the oxidation-driven size decrease of amorphous carbon nanostructures, relevant as model systems for investigations of soot oxidation. It is shown that the peak characteristics (spectral position and amplitude) are very sensitive signatures of the nanostructure size and that this can be used to accurately measure very low oxidation rates, using simple optical absorption techniques.

11:20am **NS+BI-MoM10 Microscopic and Spectroscopic Studies of Photoactive Supermolecular Porphyrin Assemblies**, ***U.M. Mazur****, K.W. Hipps, B.C. Wiggins, B.A. Friesen*, Washington State University

Synthetic porphyrins form excitonically coupled ring- and rod-shaped nanostructures, which are of interest for their potential applications in optoelectronic devices and solar cells. Nanorods produced from the sodium salt of tetrakis(4-sulfonatophenyl) porphyrin, TSPP, dissolved in acidic aqueous solutions, were deposited onto Au(111) substrates and were imaged by scanning probe microscopy. Ambient and UHV scanning tunneling microcopy, STM, images revealed individual rods with diameters of 25-40 nm and lengths of hundreds of nanometers. High resolution STM images of TSPP on Au(111) showed that the rods are composed of disk-like building blocks approximately 6.0 nm in diameter. We speculate that the disks are formed by a circular *J*-aggregation of 14-16 overlapping electronically coupled porphyrin chromophores and that this circular porphyrin organization is driven by nonplanar distortions of the porphyrin diacid. X-ray photoelectron spectroscopy, XPS, was employed to investigate the structural changes in TSPP associated with the protonation of the porphyrin ring and aggregation. Scanning tunneling spectroscopy, STS, results indicate high conductivity of TSPP nanorods that can be attributed LUMO based conductivity in the nanorods.

Our results shed light not only on the structural model for TSPP basic aggregate unit but also on the conduction mechanism within the TSPP nanorod structure.

11:40am **NS+BI-MoM11 Solid-State Tunneling Spectroscopy of Individual Nanoparticles**, ***R. Subramanian***, *P. Bhadrachalam, V. Ray, S.J. Koh*, University of Texas at Arlington

Semiconducting nanoparticles are increasingly finding innovative applications in many areas of science and technology such as bio-medicine, solar-energy harvesting, and photonics. For effective use of nanoparticles for these applications, it is necessary to know their electronic structures and efficient and accurate techniques to measure them are desired. We present a new technique to directly probe the energy levels of individual semiconducting nanoparticles in which the units to measure the electronic structures are fabricated using CMOS-compatible processes. This technique not only enables us to probe energy levels of an individual nanoparticle, but allows carrying out many such measurements from numerous units fabricated with a single-batch parallel processing. The energy levels were directly obtained with the I-V measurement through double barrier tunnel junctions that were formed when the nanoparticles were placed between vertically separated source and drain electrodes. The band gap (E_g~1.92eV) and energy level spacings (ΔE ~ 130meV, ΔE_{p-d}~ 96meV and ΔE_{d-f}~ 103meV) were measured directly from the current-voltage and differential conductance spectra for colloidal CdSe nanoparticles (~7nm). Measurements for core-shell semiconducting nanoparticles (such as InP/ZnS) will also be presented. (Supported by NSF CAREER (ECS-0449958), ONR (N00014-05-1-0030), and THECB ARP (003656-0014-2006))

Biomaterial Interfaces
Room: K - Session BI-MoA

Protein and Cell Interactions at Interfaces I
Moderator: T. Boland, Clemson University, B.G. Liedberg, Linköping University, Sweden

2:00pm **BI-MoA1 Bioengineering Stem Cell Fate**, *H.M. Blau, **K. Havenstrite***, Stanford University **INVITED**

A major challenge facing stem cell biologists is an understanding of the mechanisms that direct stem cell fate: the delicate balance between quiescence, self-renewal, and differentiation. Adult stem cells are localized in niches, specialized microenvironments, which protect them from differentiation. Upon culture, adult stem cells lose their "stemness", or ability to self-renew. We have engineered artificial in vitro microenvironments that mimic key biochemical characteristics of adult stem cell niches in order to analyze the properties of stem cells and influence their fate. Microwell arrays are produced as topographically structured polymer hydrogel surfaces allowing exposure of single cells either to soluble or tethered niche proteins. Using this platform, phenotypic and dynamic analyses of thousands of individual cells can be monitored simultaneously by time lapse microscopy. We have found that single proteins alter proliferation kinetics and asymmetric division behavior, leading to muscle and hematopoietic stem cell self-renewal in culture. Our data demonstrate that parameters of proliferation behavior in vitro correlate with stem cell function assayed in vivo. Ultimately, the goal of these studies is to increase our understanding of stem cell biology, expand stem cells in vitro for clinical applications, and discover new drugs for stimulating a patient's own stem cells.

2:40pm **BI-MoA3 An Investigation of Human Embryonic Stem Cell Attachment on 496 Different Acrylate Polymers in a Microarray: The Importance of Surface Chemistry as Probed by ToF SIMS**, *M.R. Alexander, **J. Yang**, M.C. Davies,* The University of Nottingham, UK, *Y. Mei, D.G. Anderson, R.S. Langer,* MIT, *M. Taylor, A.J. Urquhart*, The University of Nottingham, UK

The relationship between the surface chemistry of materials and human cellular response has great importance in existing and emerging technology areas such as tissue engineering, regenerative medicine and biosensors. Here, we investigate hESC attachment, surface chemistry (using time of flight secondary ion mass spectrometry (ToF SIMS) and XPS) and bulk properties (using confocal Raman spectroscopy) of a large set of samples with diverse chemistry. These are acrylate polymers in the form of micro-spots in an array made from 22 different acrylate monomers mixed pairwise in different proportions and UV photopolymerised to give 496 unique homo- and co-polymers.[1, 2] We do not find a correlation between the human embryonic stem (hES) cell number and wettability, or surface elemental or functional composition that holds for all the samples on the array. In contrast, surface mass spectrometric data acquired using ToF SIMS correlate strongly with cell attachment on all polymers using partial least squares (PLS) regression. The ability to predict cell attachment using the SIMS data indicated that it contains sufficient information on the surface chemistry of the polymers to describe the effect of surface chemistry on cell attachment. Some of the moieties identified using this approach are consistent with previous theories relating surface chemistry on protein adsorption and in turn to cell adhesion, whereas others are new.

We propose that in the field of cell-material interactions, this result highlights the importance of the molecular information contained in the SIMS spectra in controlling the cell attachment. Furthermore, it indicates how the PLS methodology can be used to identify the relationship between surface chemical moieties represented within the SIMS spectra to complex properties such as cellular response.

[1] D.G. Anderson, S. Levenberg, R. Langer, *Nature Biotechnology*, **2004**, 22, 863-866.

[2] M. Taylor, A.J. Urquhart, D.G. Anderson, R. Langer, M.C. Davies, M.R. Alexander, *Surface and Interface Analysis*, **2009**, 41, 127-135.

3:40pm **BI-MoA6 Design of Protein Polymers as Novel Tissue Engineering Scaffolds**, ***D. Sengupta**, S.C. Heilshorn*, Stanford University

The ability to tailor specific cell-matrix interactions in biomaterials is now recognized as an important method to control cell behaviour. Biomaterial adhesivity and elasticity are important determinants of cell adhesion, proliferation, and differentiation; and a coordinated cell response to these different material inputs results in complex signaling crosstalk. Independent modification of these biomaterial properties is thus extremely important, but difficult to achieve with current synthetic as well as natural biomaterials. While natural biomaterials such as collagen and Matrigel do not allow for the independent tuning of multiple biomaterial properties, synthetic biomaterials such as PEG and acrylates can be toxic and immunogenic. An alternative approach to natural as well as synthetic materials is the use of protein polymers made with recombinant protein engineering technology. By templating protein synthesis using the genetic code, we have exact molecular-level control over our material. Using this strategy, we have engineered a family of tunable and biodegradable protein-engineered biomimetic materials that incorporate critical elements of the natural extracellular matrix. The materials are manufactured using a modular design strategy, resulting in a fusion protein comprised of multiple peptide domains that provide cell adhesion and matrix elasticity. Specifically, the elastic modulus of the material can be tuned (from ~43-1200 kPa) independently of RGD ligand density (from 0-9300 cell adhesion sites/µm2), enabling optimization of the biomaterial interface for specific tissue engineering applications. Additionally, these interfaces can be easily micro-molded to incorporate micro- or nanoscale topographical features that induce cell alignment. Human embryonic stem cell-derived cardiomyocytes as well as mouse embryonic stem cells cultured on our protein-engineered biomaterials demonstrate viability, proliferation, differentiation, and morphology comparable to positive gelatin controls, providing a viable alternative to commonly used materials. The molecular-level design strategy of these protein polymers allows for unprecedented control over the biomaterial-cell interface for regenerative medicine applications.

4:00pm **BI-MoA7 Axon Guidance on Patterned Gradients of Extracellular Matrix Proteins**, *W. Theilacker, H. Bui,* University of Delaware, *S. Sullivan,* Alcoa Technical Center, *L. Capriotti,* University of Delaware, *D. Willis, J. Twiss,* Alfred I. duPont Hospital for Children, *N. Zander,* Army Aberdeen Research Laboratory, *Z. Zhang,* Excellatron, ***T. Beebe Jr***, University of Delaware

This presentation will focus on axonal extension experiments made possible by recent developments in a general platform for substrate patterning of protein and peptide gradients using covalent attachment schemes, and employing cell- and protein-resistant lanes of PEO-like comb polymer. The platform uses step gradients and continuous gradients in local protein and peptide concentrations from micron to centimeter length scales. Control of the local surface density of proteins and peptides allows cell culture assays involving competition of cells for different extracellular matrix (ECM) proteins, propensity of axons to cross from one ECM protein lane into a different ECM protein lane, neuron attachment propensity, axon extension direction and rate, and controlled studies of cell-cell interactions between different cell types. This presentation will address the relationship between the local protein coverage and the "bioactivity" or "bioavailability," using a variety of surface analytical techniques including XPS, TOF-SIMS and AFM, and optical microscopy techniques including epi- and confocal fluorescence microscopy.

4:20pm **BI-MoA8 Aligned Highly Porous Electrospun Scaffolds for Nerve Tissue Engineering**, ***N. Zander**,* Army Research Lab/University of Delaware, *J. Orlicki, A. Rawlett,* Army Research Lab, *T. Beebe*, University of Delaware

Spinal cord injuries are one of the most catastrophic and costly types of injuries since damaged axons in the central nervous system are unable to spontaneously regenerate. Although reconstruction of damaged and diseased neural pathways remains a major hurdle, recent research has shown that aligned electrospun fiber mats can provide contact guidance cues to direct axon growth by acting as a bridging device. However, due to the nanometer sized fiber diameter and highly aligned nature of the scaffolds, the low interfiber distance limits penetration of the cells into the scaffold.

To study the effect of fiber mat porosity on cellular infiltration, aligned fiber mats were fabricated via co-electrospinning polycaprolactone with polyethylene oxide (a water soluble polymer). Variation of the fabrication parameters allowed for control of the porosity of the scaffold with a full range of sacrificial (PEO) fiber composition. As the surface composition is also critical in providing biochemical signals to direct neurite growth, the surfaces of the fibers were functionalized via air plasma treatment followed by attachment of several extracellular matrix proteins. The surface chemistry was characterized by X-ray Photoelectron Spectroscopy, Time of Flight Secondary Ion Mass Spectrometry, and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy. Cellular infiltration, proliferation and neurite outgrowth of PC12 cells were evaluated for fiber mats of varying porosity and surface composition.

4:40pm **BI-MoA9 Study of GPIbα and vWF Interations under Blood Flow**, ***X. Cui***, *HB. de Laat, J. Orje, Z.M. Ruggeri*, The Scripps Research Institute

The mechanism of platelet-adhesion plays an important role in hemostasis and thrombosis. When blood vessels are injured or disrupted, the platelets membrane presented glycoprotein Ibα (GPIbα) will bind to von Willebrand Factor (vWF), which is a large multimeric plasma protein immobilized on collagen fibers. The binding interactions are regulated by the applied fluid shear rates. In this paper, we conducted a study of blood platelets adhesion on vWF A1 domain coated glass slides at different shear rates. The platelets movements are recorded by a high speed camera at 30fps. The recorded videos are analyzed using video/image processing software in order to calculate the platelet velocities at different shear rates as well as different protein coating concentrations. We found the average velocity decreased when the shear rate increased. This showed the binding requires high fluid shear rate in the flow. PLGA beads with the shapes of ellipsoid and sphere of about 1µm diameter were also studied using this method. However, the beads were coated with vWF A1 domain and the flow chambers were coated with GPIbα. We observed the decrease of the velocity with the increased shear rates, which showed increased binding strength between vWF A1 domain and GPIbα protein at higher shear rates. The ellipsoid beads had lower velocity comparing with the sphere beads at the same shear rate due to the larger contact area to the coating surface.

5:00pm **BI-MoA10 Platelets Adhere to Adsorbed Albumin through a Receptor-Mediated Process**, ***B. Sivaraman***, *R.A. Latour*, Clemson University

Since albumin (Alb) lacks known sequences recognized by platelet receptors, it is not supposed to support platelet adhesion. However, studies have suggested that platelets may be able to adhere to adsorbed Alb (*1, 2*) with adhesion related to adsorption-induced Alb unfolding (*1*), although the mechanisms of this remain unclear. To address this issue, we conducted studies to definitively determine if platelets adhere to adsorbed Alb, whether adhesion is related to adsorbed Alb conformation,and if it occurs by a receptor-mediated process. Alb was adsorbed at 0.1, 1.0, and 10 mg/mL on various alkanethiol SAM surfaces to vary the degree of unfolding in the adsorbed Alb. The adsorption-induced conformational changes in Alb was quantified by CD spectropolarimetry (*3*). Platelet adhesion studies were carried out and the platelet response determined by LDH assay and SEM. A series of platelet adhesion inhibitors and protein modification agents were used to probe the mechanisms of platelet adhesion. Platelet adhesion to adsorbed Alb was negligible when adsorbed Alb retained most of its native structure (< 34% loss in alpha-helix), but began to linearly increase with the degree of adsorption-induced unfolding thereafter (r^2=0.92). SDS-PAGE results showed that the platelet suspension was free of residual proteins and anti-Alb polyclonal antibodies completely inhibited platelet adhesion to adsorbed Alb, but had negligible effect on adsorbed Fg (used as a control); thus confirming that the platelets were adhering to adsorbed Alb and not some other residual protein in the system. Addition of an RGDS peptide to the platelet suspension strongly inhibited platelet adhesion to adsorbed Alb (~60% reduction on CH_3 SAM; $p < 0.01$), while the addition of RGES peptide had no inhibitory effect. Neutralization of arginine residues in the adsorbed Alb layer using 2,3-butanedione reduced platelet adhesion to a similar degree as exposing the platelets to the RGDS peptide in solution. These results indicate that the adhesion of nonactivated platelets to adsorbed Alb is primarily mediated by RGD-specific receptors and the degree to which the binding domains in adsorbed Alb are exposed and/or formed is directly proportional to the degree of adsorption-induced unfolding of the protein. Further studies will be carried out to identify the specific platelet receptors and Alb domains that mediate adhesion. These results indicate that we have much yet to learn about the mechanisms that influence platelet adhesion to adsorbed proteins.

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Biomaterial Interfaces
Room: K - Session BI-TuM

Proteins and Cell Interactions at Interfaces II
Moderator: H.E. Canavan, University of New Mexico, S.L. McArthur, Swinburne University of Technology, Australia

8:00am **BI-TuM1 Molecular Simulation as a Surface Design Tool to Understand and Control Protein-Surface Interactions**, ***R.A. Latour***, Clemson University **INVITED**

Protein-surface interactions are of great importance in a wide variety of applications in biomedical engineering and biotechnology, including medical implants, biocatalysis, immobilized-enzyme bioreactors, biosensors, bioseparations, and bioanalytical systems. While this is well recognized, very little is understood regarding how to design surfaces to optimally control protein adsorption behavior. To address this limitation, we are working on the development of molecular simulation methods to accurately predict protein-surface interactions at the atomic level. We have found that this type of molecular system is sufficiently unique that molecular simulation methods cannot simply be borrowed from other applications; but rather, they must be critically evaluated and often modified to accurately represent adsorption behavior. In this talk, I will address four major areas that we have identified as being particularly important for the simulation of protein-surface interactions, and I will present our approaches to address each of these areas. These are (1) the general methods that are needed to properly simulate protein-surface interactions, (2) the suitability of a force field to represent protein-surface interactions, (3) the adequate treatment of solvation effects, and (4) the need for advanced sampling methods for large molecular systems. I will present an overview of our efforts to address each of these key areas. We are developing a hybrid force field program that enables multiple force fields to be used in a single simulation to represent different phases of a system (e.g., solid surface, solution, and the interphase between them), methods to enable pressure to be properly monitored and controlled in a simulation with constrained atoms, and how electrostatic effects should be represented for surfaces with high charge density when using periodic boundary conditions. We have generated a large experimental benchmark data set for peptide-surface interactions for use for force field evaluation, modification, and validation purposes along with simulation methods to calculate adsorption free energy for comparison with this data set. Regarding solvation effects, we have found that existing implicit solvation methods are completely unsatisfactory at this time and must be redeveloped before use in protein adsorption simulations. Finally, we are also developing advanced sampling methods for large molecular systems to efficiently overcome energy barriers that often cause simulations to become trapped in local low-energy states and prevent proper exploration of the relevant phase space of the molecular system.

8:40am **BI-TuM3 Surface-Induced Changes in the Structure of Beta-Helical Peptides**, ***K. Fears***, *J. Kulp III, D.Y. Petrovykh, T. Clark*, US Naval Research Laboratory

The stable structure of beta-helical peptides in solution provides a well-defined starting point for discerning the changes in secondary structure of peptides induced by surface adsorption. Understanding the adsorption of proteins on surfaces is of critical importance in medical- and bio-technology. The determination of the secondary and higher-order structure of adsorbed proteins, however, is challenging due to their inherent complexity. Peptides with simple secondary structures provide a good model for investigating the interactions between surfaces and the structural subunits of proteins. Beta-helical, rather than alpha-helical, peptides were selected for this study because of their stability and tendency to maintain a monomeric, unaggregated structure. Peptides were custom designed to switch between two different stable conformations as a function of solvent composition, as confirmed by nuclear magnetic resonance (NMR) and circular dichroism (CD) spectroscopies in solution. These peptides were adsorbed onto silica substrates from aqueous and organic solvents to determine their conformation post-adsorption. The secondary structure assignments of the adsorbed peptides were based on the comparison of their CD spectra to spectra of the same peptides in their known solution conformation. Our ability to measure the secondary structure of peptides that are adsorbed as monolayers on planar substrates suggests that CD spectroscopy can be used to gain insight about the adsorption behavior of individual subunits that provide support and/or functionality to proteins.

9:00am **BI-TuM4 Enantiospecific Adsorption of Serine Enantiomers on the Chiral Cu{531} Surface**, *T. Eralp, A. Shavorskiy,* University of Reading, UK, *D. Batchelor,* BESSY and Universität Würzburg, Germany, ***G. Held***, University of Reading, UK

The production of enantiopure chiral bio-relevant molecules is of significant importance for the development of new pharmaceuticals and the improvement of existing ones. In this context chiral surface systems, e.g. chiral molecules adsorbed on chiral single crystal surfaces, are of considerable interest as they are model systems for potential enentioselective heterogeneous catalysts or enantiomeric selection. In this study the adsorption properties of L- and D- Serine enantiomers on the intrinsically chiral Cu{531} surface were investigated. These aminoacids have four functional groups which can make bonds to the Cu{531} surface: OH, NH_2, and two oxygen atoms in the carboxyl group (-COOH). The geometry of the adsorption complex was characterised using XPS and NEXAFS. The bonding characteristics of the molecule strongly depend on the coverage. The main peak in the O1s XPS spectra, at BE 531.5eV, is assigned to be overlapping signal of the two oxygen atoms in the deprotonated carboxylate (COO) group forming bonds with Cu atoms. For the low coverages this peak has a shoulder at a lower BE (530.7 eV), as the coverage increases this shoulder disappears and a new peak appears at higher BE (532.8 eV). The low BE shoulder at low coverage is assigned to the OH group also forming a bond with the Cu surface. With increasing coverage the surface becomes more crowded and a less space-consuming configuration is assumed with a 'dangling' OH group, which is the origin of the high BE O1s peak.

In order to investigate the orientation of the amino acids within the surface NEXAFS spectra were recorded for different in-plane polarization angles and different coverages. These spectra show large enantiomeric differences between the orientation of D-serine and L-serine, which is also reflected in the LEED patterns, indicating different long-range ordered overlayers. These enantiomeric differences are much bigger than those previously observed for alanine on the same surface, which suggests that the size and the nature of the amino acid side group is the dominating factor for enantioselective behaviour on this surface.

9:20am **BI-TuM5 Investigation of Surface-Bound Protein Conformation/Orientation Using Time-of-Flight Secondary Ion Mass Spectrometry**, ***L.J. Gamble***, *F. Cheng, J. Brison, L. Árnadóttir, D.G. Castner*, University of Washington

The adsorption of protein to solid surfaces is typically accompanied by structural rearrangements as well as loss of bioactivity. These changes can be monitored by time-of-flight secondary ion mass spectrometry (ToF-SIMS) and the protein activity monitored by surface plasmon resonance (SPR). However, the ultra-high vacuum of the ToF-SIMS can alter the protein conformation. In this study ToF-SIMS was coupled with a variable temperature sample stage to monitor the conformational changes that occur when a surface-bound protein goes from a hydrated to a dehydrated state. Changes in bioactivity of the surface bound proteins were investigated using SPR. Initial ToF-SIMS and SPR experiments were conducted on a surface-bound protein system of histagged humanized anti-lysozyme variable fragment (HuLys Fv) coordinated on a Ni^{2+}-loaded nitrilotriacetic acid (NTA) surface. Continuing studies investigate fibrinogen (and fibrinogen fragments). Positive ToF-SIMS data from the protein surfaces were acquired with an ION-TOF TOF.SIMS 5-100 system (ION-TOF GmbH, Münster, Germany) . Applying principal component analysis (PCA) to the ToF-SIMS data, the spectral differences resulting from two surface coverages and various heat treatments were determined. The spectra are separated into three groups: high protein coverage samples, low coverage below -80°C, and low coverage at -60°C and above. Trends observed in the plot suggest both surface coverage and heat treatment affected the secondary ion spectra. At the temperature below about -80oC, the protein molecules are frozen into their hydrated conformation. As the temperature is raised changes expose hydrophobic amino acid residues. The antigen binding capacity of surface-bound HuLys Fv before and after dehydration was measured by SPR. At the low coverage, the antigen binding capacity on the dried protein film was roughly 50% lower than that on the fresh film. As comparison, high coverage dried samples lost ~20% binding capacity. The loss of HuLys Fv bioactivity on the dried protein film was attributed to an irreversible disruption of protein native conformation during the drying process. The high coverage samples exhibited less loss of bioactivity, consistent with the smaller conformational changes observed by PCA. The use of the sugar trehalose as a protein stabilizer is also investigated.

9:40am **BI-TuM6 Soft X-ray Spectromicroscopy of Protein Interactions with Model Biomaterials**, ***B.O. Leung**, A.P. Hitchcock, J.L. Brash,* McMaster University, Canada, *A. Scholl, A. Doran*, Advanced Light Source

Upon implantation in biological tissue or first contact with blood, all materials are immediately coated with a layer of proteins. The details of this initial protein layer can have a very strong effect on biocompatibility [1]. Thus, characterization of the surfaces of biomaterials and their interaction with relevant proteins can help to determine and understand biocompatibility.

We use synchrotron based X-ray photoemission electron microscopy (X-PEEM) [2] and scanning transmission X-ray microscopy (STXM) [3] to study the spatial distribution of adsorbed proteins on chemically heterogeneous surfaces. Both techniques have a lateral spatial resolution below 40 nm, provide speciation and quantitation through spatially resolved near-edge X-ray absorption fine structure (NEXAFS) spectroscopy, and can be used to obtain quantitative maps of the adsorbed protein in relation to the topography and chemistry of the underlying substrate with high sensitivity [4-5]. X-PEEM probes the top 10 nm of the sample whereas STXM integrates over the full sample thickness (40-100 nm) and can be applied to fully hydrated samples.

Results from three recent studies will be presented. (1) spun-cast thin films of phase-segregated polystyrene blended with cross-linked polyethylene oxide (PEO) adsorb human serum albumin (HSA) more readily to the PEO -rich areas compared to plasma-deposited diglyme surfaces, which are known protein resistant materials. Due to incomplete phase segregation, PEO imparts some protein resistance to the PS region. (2) HSA adsorption to polystyrene/ poly(methyl methacrylate)-b-polyacrylic acid (PS/PMMA-b-PAA) thin films show strong modifications of the adsorption behavior relative to HSA adsorption to PS/PMMA surfaces due to strong and specific electrostatic interactions between the positively charged peptide and the negatively charged PMMA-b-PAA domains. (3) Protein adsorption to PS-PMMA spun-cast films has been examined by STXM in a fully hydrated wet cell. In the presence of water, HSA preferentially adsorbs to the discrete polar PMMA domains rather than the continuous PS domain, whereas the latter is favored under dried conditions [2]. [6]

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6. Research carried out with PEEM2 and STXM532 at the Advanced Light Source, which is supported by Basic Energy Sciences, DoE, USA. Research funded by NSERC.

10:40am **BI-TuM9 Probing Individual Side-Chains of Peptides at Interfaces Using Isotope Labeling with Sum Frequency Generation Spectroscopy**, ***T. Weidner**, N.F. Breen, G.P. Drobny, D.G. Castner*, University of Washington

Controlled immobilization of peptides onto artificial biointerfaces plays a key role in antifouling, implant and immunosensor technologies and it is of crucial importance to develop tools to examine interfacial properties of adsorbed peptides. Sum frequency generation (SFG) spectroscopy can probe biomolecules at the solid-liquid interface. Isotope labeling can address specific protein regions but its potential in conjunction with SFG spectroscopy has remained mostly unexplored. We combine these techniques to characterize the structure of synthetic model peptides on surfaces in PBS buffer. The peptide used is a 14-mer of hydrophilic lysine (K) and hydrophobic leucine (L) residues with an α-helical secondary structure. These LK14 peptides have the hydrophobic side-chains on one side of the helix and the hydrophilic on the other. Deuteration of the isopropyl group of each of the leucines, totaling 8 samples, was used to probe individual leucine side chains of LK14 adsorbed onto a hydrophobic polystyrene surface. Side-chain orientations were determined using ratios of the asymmetric CD_3 stretching mode at 2221 cm^{-1} acquired with different polarization combinations. We found that the orientation of the leucine side chains in the surface-bound LK14 was remarkably different from the calculated solution structure. Leucines in the center of the peptide are more oriented towards the surface while those at the ends of the amino-acid sequence are more bent away, indicating the leucines in the center of the peptide chain play a dominant role for the binding of the peptide. Solid state NMR data acquired on polystyrene beads is in line with the determined orientation change upon binding. In addition, ^{15}N labeling was used to address the controversial assignment of a pronounced peak near 3300 cm^{-1} observed for a variety of proteins adsorbed onto surfaces. This spectral feature has been assigned to both N-H containing side chains and backbone-related amide A resonances before. ^{15}N labeling of the lysine side chains resulted in a 9 cm^{-1} red-shift of this peak in the spectrum of LK adsorbed onto a SiO_2 surface, showing the 3300 cm^{-1} feature is related to the terminal amine group on the lysine side chains.

11:00am **BI-TuM10 Surface-initiated Vapor Deposition Polymerization of Poly(gamma-benzyl L glutamate): Optimization and Mechanistic Study**, ***W. Zheng**, C.W. Frank*, Stanford University

Surface-initiated vapor deposition polymerization (SI-VDP) is a highly effective approach to synthesize grafted polypeptides. In this study, we developed an SI-VDP system having pressure and temperature control to reduce vacuum restriction 1000 times with high grafting efficiency and, thus, were able to synthesize grafted poly(gamma-benzyl L-glutamate)(PBLG) film of 167nm thick under 0.75 mbar. More importantly, we quantitatively investigated mechanistic details of the SI-VDP process including monomer vaporization and reservoir polymerization in the monomer reservoir and monomer condensation and physisorbed and chemisorbed polymerization on the substrate surface. To study the major monomer reservoir processes, we monitored the amount of vaporized monomers and developed a VDP reaction profile (VDPRP) method. We found that the VDPRPs were mostly contributed by the reservoir processes. We also found that characteristic features of the VDPRPs were determined by the monomer heating temperature and proposed possible mechanisms for the feature evolution. To evaluate the major substrate surface processes, we developed a quantitative analysis method using FTIR on both as-deposited PBLGs and chemisorbed PBLGs. Consequently, we were able to propose possible SI-VDP mechanisms leading to the surface-grafted PBLGs that were expected to have either high packing density with mostly α-helix segments or low packing density with a significant amount of both random coil and α-helix segments.

11:20am **BI-TuM11 Adsorbed α-Helical Polypeptides: Molecular Organization, Structural Properties, and Interactions**, ***B. Atmaja***, Stanford University, *J.N. Cha,* University of California, San Diego, *C.W. Frank*, Stanford University

In this work, we have developed 11-mercaptoundecanoic acid (MUA)-polypeptide "bilayer" systems by adsorbing poly(diethylene glycol-L-lysine)-poly(L-lysine) (PEGLL-PLL) diblock copolypeptide molecules of various architectures onto MUA-functionalized gold substrates. Previously, we reported the self-assembly of PEGLL-PLL with nanoparticles that were functionalized with carboxylic acid (COOH) moieties to form a variety of supramolecular structures. In relation to this previous work, we have used the PEGLL-PLL/MUA bilayer as a model system for studying the interfacial phenomena that occur when the PEGLL-PLL molecules interact with the COOH moieties of nanoparticle ligands. Specifically, we have elucidated the nature of the interactions between the PEGLL-PLL and COOH moieties as well as the resulting polypeptide conformation and organization, using a combination of surface techniques—grazing-incidence infrared (IR) spectroscopy, ellipsometry, and contact angle. Because our PEGLL-PLL/MUA bilayer system can potentially be applied as a nonfouling surface, we have thoroughly characterized other film properties such as the packing and graft density of the polypeptide molecules as a function of the PEGLL-PLL architecture. A complete understanding of the film's molecular structure would then allow us to elucidate the relationship between the bilayer's nonfouling characteristics and its underlying structure in our future work. Using IR spectroscopy, the adsorption process is determined to occur primarily by means of electrostatic interaction between the protonated PLL residues (pKa ~ 10.6) and carboxylate moieties of the MUA SAM (pKa ~ 6) that is enhanced by H-bonding. The PLL block is thought to adopt a random-coil (extended) conformation, while the PEGLL block that is not interacting with the MUA molecules is found to adopt an α-helical conformation with an average tilt-angle of ~ 60°. The PEGLL-PLL molecules have also been deduced to form a heterogeneous film and adopt a liquid-like/disordered packing on the surface. The average contact angle of the polypeptide/MUA bilayer systems is ~ 40°, which implies that the diethylene glycol (EG2) side chains of the PEGLL residues may be oriented somewhat toward the surface normal. From ellipsometry measurements, it is found that PEGLL-PLL molecules with a longer α-helical block are associated with a lower graft density on the MUA surface compared to those with a shorter α-helical block. This observation may be attributed to the greater repulsion—steric and H-bonding effects—that is imposed by the EG2 side chains found on and projected area occupied by the longer PEGLL block.

Biomaterial Interfaces
Room: K - Session BI-TuA

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

2:00pm **BI-TuA1 Anti-Fouling Hydrogels for Biomaterials and Sensing Applications**, ***B.G. Liedberg***, Linköping University, Sweden **INVITED**

The present contribution describes a novel set of hydrogel coatings prepared by self-initiated photografting and photopolymerization (SIPGP). The method is based on UV free radical polymerization of methacrylate and acrylate monomers into 0-200 nm thick coatings on top of virtually any organic/polymeric substrate. The vast majority of the coatings are based on different mixtures of PEG containing methacrylates. These hydrogels display excellent protein rejecting properties in simple single component solutions and mixtures, as well as in serum and plasma [1]. Recent studies on platelet adsorption looks also were promising. The UV fabrication technology is also compatible with patterning on the micrometer length scale, thus providing an attractive platform for biochip development and microarraying [2]. The preparation of gradients is also discussed for tuning the physio/chemical properties of the hydrogel coatings [3]. A novel set of charged balanced hydrogel gradients have been prepared for studies of protein adsorption from protein mixtures and plasma with encouraging results [4]. Finally we describe an approach for array fabrication of nanobrushes/bushes on a pre-patterned template generated by Dip Pen Nanolithography (DPN).

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2:40pm **BI-TuA3 Emerging Strategies to Prevent Bacterial Colonization of Medical Biomaterials**, ***J.D. Bryers***, University of Washington **INVITED**

Nosocomial (hospital-acquired) infections are the fourth leading cause of death in the U.S. with >2 million cases annually (or ~10% of American hospital patients). About 60-70% of all such infections are associated with an implanted medical device causing >$4.5 billion medical costs in 2002 and ~99,000 deaths annually. Over 65% of hospital-acquired infections are associated with implants or indwelling medical devices, with the case-to-fatality ratio between 5-50%. It is estimated that over 5 million artificial or prosthetic devices are implanted per annum in the U.S. alone. Microbial infections have been observed on most biomedical devices, including: prosthetic heart valves, orthopedic implants, intravascular catheters, artificial hearts, left ventricular assist devices, cardiac pacemakers, vascular prostheses, cerebrospinal fluid shunts, urinary catheters, ocular prostheses and contact lenses, and intrauterine contraceptive devices.

Traditional strategies to control medical device-based biofilm infections are based on the use of compounds that kill or inhibit the growth of *freely suspended* bacteria. However, “biofilm-bound” bacteria tend to be significantly less responsive to antibiotics and antimicrobial stressors than planktonic organisms of the same species. In fact, studies have shown that sub-lethal doses of antibiotics can exacerbate biofilm formation. Consequently, systemic antibiotic treatment typically fails to clear a biofilm infection and inevitably requires removal of the device. Moreover, the risk of antibiotic resistance development is drastically increased under the current standard use of systemic antibiotic treatment of medical-device infections.

Here novel non-antibiotic based concepts in biomaterials design (novel stealth surfaces or biomaterials that biologically prevent bacterial colonization) will be presented.

4:00pm **BI-TuA7 Protein Adsorption - Influence from Surface and Protein Characteristics**, ***M. Holmberg***, *X. Hou*, Technical University of Denmark

Competitive protein adsorption from human serum and protein mixtures onto unmodified and plasma polymerised polymer surfaces has been investigated using radioactive multi-labelling. By using several different isotopes it is possible to monitor several proteins simultaneously and thereby analyse the interaction between the different proteins during adsorption processes.

The outcome of competitive protein adsorption depends on both surface and protein characteristics, as well as parameters such as protein concentration and adsorption sequence. In this study surface characteristics of polymer surfaces have been modified by using plasma polymerisation where the polymer surface becomes more hydrophilic. Modified surfaces show non-fouling characteristics and have shown to be stable in buffer solutions for at least 24 hours. By changing the sequence of proteins introduced to a surface, different outcome from an adsorption series with the same proteins can be observed and by changing the internal ratio between different proteins concentration, different proteins will dominate the surface during adsorption.

Even though some polymer surfaces show protein monolayer adsorption behaviour and quite low amount of proteins adsorbed, other surfaces loose there resistance to protein adsorption as the protein concentration increases, and on hydrophobic polymer surface one can even see a very thick and cross linked protein multilayer formed. The tendency for protein multilayer formation is also influenced by other proteins present during adsorption and protein characteristics, where some proteins seem to be more fragile during adsorption to hydrophobic polymer surfaces than others.

The objective of the study is two-fold; to investigate basic processes and concepts during competitive protein adsorption and to contribute to development of polymer based biomaterials for use in contact with whole blood.

4:20pm **BI-TuA8 Influence of Physicochemical Surface Properties on the Adhesion of Marine Microorganisms**, ***A. Rosenhahn***, *S. Schilp, X. Cao, F. Wode, M.P. Arpa Sancet, M. Heydt, M. Grunze*, University of Heidelberg, Germany

The prevention of biofouling is a major challenge for all manmade objects which are in long term contact with seawater. In order to systematically develop non toxic coatings, a fundamental understanding of basic surface properties relevant for adhesion of marine inhabitants is required. To determine the influence of selected surface properties we systematically vary wetting, hydration and charge by self assembly of oligo- and polymers. To obtain well defined morphologies, nanolithography and multilayer assembly are used. The biological response is determined in settlement and adhesion strength assays using predominantly the green algae Ulva linza, but also barnacle cyprids and marine bacteria. It turned out that contact angles around the Berg limit, hydration of the coatings and micrometer sized structures render surfaces less attractive. Besides static assays we are interested in the time dependent dynamics of biofilm formation. To acquire and analyze the complex, 3D swimming and exploration patterns of algal zoospores, we apply digital in-line laser holography. The influence of surface properties on the motion patterns and surface recognition will be discussed.

4:40pm **BI-TuA9 *In situ* Characterization of Barnacle Primary Cement Interfaces by ATR-FTIR Spectroscopy**, ***D.E. Barlow***, U.S. Naval Research Laboratory, *G Dickinson, B. Orihuela, D. Rittschof,* Duke University Marine Laboratory, *K.J. Wahl*, U.S. Naval Research Laboratory

Understanding the chemistry of barnacle adhesion is of great interest in the areas of marine biofouling prevention and materials science of adhesives. Barnacles adhere to surfaces by a proteinaceous cement, for which most studies to date have been *ex situ* analyses of the protein composition. However, very little is currently known about the chemical structure and composition in the original, undisturbed cement interfaces of barnacles (primary cement interfaces) that provide the strong adhesion to substrates in marine environments. We will present a method that has been implemented for characterizing primary cement interfaces of barnacles using *in situ* attenuated total reflection - Fourier transform infrared spectroscopy (ATR-FTIR). Primary cement of the barnacle *Balanus amphitrite* (= *Amphibalanus amphitrite*) was characterized without any disruption to the original cement interface, after settling and growing barnacles directly on double side polished germanium wafers. High quality IR spectra were acquired of live barnacle cement interfaces, providing a spectroscopic fingerprint of cured primary cement *in vivo* with the barnacle adhered to the substrate. Additional spectra were also acquired of intact cement interfaces for which the upper portion of the barnacle had been removed leaving only the base plate and cement layer attached to the substrate. This allowed further characterization of primary cement interfaces that were dried or placed in D 2 O. The resulting spectra were consistent with a proteinaceous cement, and allowed analysis of the protein secondary structure and water content in the cement layer. The estimated secondary structure composition was primarily b-sheet, with additional a-helix, turn, and unordered

components. The cement of live barnacles, freshly removed from seawater, was estimated to have a water content of 20% - 50% by weight. These results provide new insights into the chemical properties of the undisturbed barnacle adhesive interface. The ATR-FTIR method presented is also expected to be useful for *in situ* and *in vivo* studies of bioadhesives from other organisms.

Biomaterial Interfaces
Room: Hall 3 - Session BI-TuP

Biomaterial Interfaces Poster Session I

BI-TuP2 Formation of Stable Microbubbles by Encapsulation in Silica, ***K. Staggs***, University of New Mexico, *G. Gupta*, Los Alamos National Laboratory, *M. Tartis*, New Mexico Tech, *G.P. Lopez*, University of New Mexico

A new technique for stabilization of supramolecular assemblies (termed soft petrification) has been applied to the stabilization of microbubbles. Commonly used as ultrasound contrast agents, most types of microbubbles are inherently unstable. Current procedures require many types of microbubbles to be filled with hydrophobic, high molecular weight gases such as octafluoropropane and perfluorobutane. Special equipment including syringe pumps and sealing devices are required, because most microbubbles must be formed under these types of gases. This requirement limits the conditions under which microbubbles can be studied. They cannot be studied on a bench top open to atmosphere. Once microbubbles have undergone soft petrification, they can be studied in a wide range of environments. In soft petrification of air filled lipid coated microbubbles, a vapor deposition technique encapsulates the microbubbles in a thin silica shell. After undergoing soft petrification, it was observed that microbubble stability under adverse conditions is significantly improved. These adverse conditions include pressures up to 120 psig and temperatures up to 80°C. Those values are double the values found for microbubbles without the silica shell. Other unique characteristics of these microbubbles include the ability to withstand temperatures well below freezing, without loss of size. Air filled microbubbles have a bench top life span of approximately four hours before encapsulation. Following encapsulation, it is observed that the same microbubbles have a bench top lifespan of up to several months. Several formulations were characterized and unprecedented air filled microbubble studies are facilitated by this technique.

BI-TuP3 Synthesis of Grafted PNIPAAm Surfaces Using ATRP in Presence of Air for Cell Adhesion Studies, ***P. Shivapooja***, *L.K. Ista, S. Mendez, G.P. Lopez*, University of New Mexico

Poly(N-isopropylacrylamide) (PNIPAAm) is a thermoresponsive polymer that exhibits a change in relative hydrophobicity above and below its lower critical solution temperature (LCST ~ 32^0C). This stimuli responsive polymer when grafted onto surface can act as a biofouling resistant coating as it has the property of formation and collapse of hydrogen bonding with water molecules below and above the LCST. Atom transfer radical polymerization (ATRP) has been proven to yield well-defined polymer but in rigorously deoxygenated environment, for example in a Schlenk line or glove box. Matyjaszewski *et al.* group developed activators regenerated by electron transfer (ARGET) that allow ATRP in presence of limited amount of air *[Langmuir 2007, 23, 4528-4531]*. We report grafted PNIPAAm brushes synthesized by this relatively simple method of ARGET ATRP which does not require an oxygen-free environment using a small amount of reducing agent together with a catalyst under homogeneous conditions. We evaluated the effect of the amount of reducing agent and reuse of monomer solution on the grafting thickness. The grafted PNIPAAm surfaces have been characterized by FTIR, XPS, contact angle measurements and ellipsometry. We anticipate using these grafted PNIPAAm surfaces for cell adhesion studies above and below the LCST.

BI-TuP4 Undercovering the Extracellular Matrix with Thermoresponsive Microgels, ***J.A. Reed***, University of New Mexico, *R.K. Shah, T. Angelini, D.A. Weitz,* Harvard University, *H.E. Canavan*, University of New Mexico

The extracellular matrix, or ECM, remains a hidden biological interface between a cell and a substrate. One method for examining proteins, such as those in the ECM, is flow cytometry (FC), which is a fast, high throughput method of quantification. Since FC is a solution technique, it is necessary to dissociate the cells from the surface. Traditional cell harvesting methods, such as enzymatic digestion and physical scraping, damage the ECM proteins as well as the cell morphology. Recently it has been shown that a thermoresponsive polymer, poly(N-isopropyl acrylamide) or pNIPAM, can be used to harvest a sheet of cells without damaging the integrity of the ECM. Above its lower critical solution temperature (LCST), this polymer is relatively hydrophobic, and mammalian cells grown on pNIPAM-grafted surfaces act in a similar fashion as those grown on typical tissue culture polystyrene (TCPS): they proliferate into a confluent sheet. Below the LCST (i.e., room temperature) the polymer physically changes, becoming more hydrophilic and swelling. Furthermore, when the temperature of the cell culture is dropped below the LCST of the polymer, the cells will detach as an intact cell sheet. These intact cell sheets, however, are not ideal for investigation in a flow cytometer, which requires individual cells for quantification. In this work, we developed a novel method for single cell detachment using pNIPAM microgels on the order of 20-200 micrometers. After fabrication, these gels were examined to ensure pNIPAM's characteristic thermoresponse was maintained, followed by cell culture on the gels. From these results, we conclude that these microgels have the capability of giving us access to this buried biological

BI-TuP5 Facile C-terminal Attachment of Proteins on Surfaces by Hydrazine-Intein Chemical Reactions, ***P. Yang***, *S. Marinakos, A. Chilkoti*, Duke University

Protein immobilization on surfaces is important in many areas of research, including their biophysical characterization, affinity chromatography, and heterogeneous immunoassays. We describe a new site-specific C-terminal immobilization of proteins on surfaces to control the orientation and attachment point of the protein. Our strategy was to exploit intein chemistry and the related hydrazine attack reaction. To provide-proof-of principle of this approach, a tripartite thioredoxin-intein-elastin-like polypeptide (ELP) fusion was synthesized in E. coli from a plasmid-borne gene. In this fusion protein, the C-terminus of the target protein thioredoxin was fused with the N-terminus of an intein, a self-splicing protein domain. An ELP was fused to the C-terminus of the intein to facilitate purification of the fusion protein because ELP fusions allow simple purification of proteins from cell lysate by isothermal, salt-triggered phase transition of the ELP. Incorporation of the intein at the C-terminus of the target protein provided an unnatural peptide bond between the target protein and the intein, which is selectively reactive towards nucleophiles such as hydrazine. In order to immobilize the target protein –thioredoxin– a hydrazine-functionalized surface on a protein-resistant background was fabricated by modifying a gold-coated glass substrate with a mixed self-assembled monolayer consisting of a hydrazine-terminated hexa(ethylene glycol) thiol and a tri(ethylene glycol)thiol. We demonstrate that the hydrazine groups on the surface were able to directly attack the unnatural peptide bond between the target protein and the intein, liberating the thioredoxin from the tripartite fusion and covalently attaching it to the surface. As a result, the target protein was site-specifically immobilized on the planar substrates with uniform orientation. This technique provides many advantages over other site-specific immobilization methods, including faster reaction kinetics, higher surface density, and the ability to easily purify the protein prior to immobilization though a simple, one-step non-chromatographic process that exploits the phase transition of the ELP.

BI-TuP6 Fabrication and Characterization of Non-toxic and Non-Biofouling Plasma-Polymerized Polyethylene Glycol Thin Films, ***C. Choi***, *K.S. Kim, D. Jung*, Sungkyunkwan University, Rep. of Korea, *D.W. Moon, T.G. Lee*, KRISS, Rep. of Korea

Polyethylene glycol (PEG) is a key molecule in the fabrication of non-biofouling surface for various biological applications such as biochips and tissue engineering. In particular, plasma-polymerized PEG (PP-PEG) thin films have many practical uses due to their strong adhesion onto any solid substrates. In this work, we fabricate PP-PEG thin films by using the capacitively coupled plasma chemical vapor deposition (CCP-CVD) method and non-toxic PEG200 molecules as a precursor. The surfaces of the PP-PEG thin films were characterized by using contact angle measurement, X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), Fourier transform infrared (FT-IR) spectroscopy and time-of-flight secondary ion mass spectrometry (TOF-SIMS). Our results show that PP-PEG thin film surfaces deposited at low plasma power were very similar in chemical composition to the PEG polymer surfaces. In addition, these PP-PEG surfaces showed excellent non-biofouling property and biocompatibility during *in vitro* and *in vivo* tests. These results indicate that our PP-PEG thin films would be useful for practical biological applications.

BI-TuP7 Optimization of Elastin-Like Polypeptide Fusions for the Purification of Membrane Proteins by Inverse Transition Cycling, ***T. Thapa***, *S. Simons, E. Chi,* University of New Mexico, *A. Chilkoti,* Duke University, *G.P. Lopez*, University of New Mexico

Low column efficiency is a common problem associated with the affinity purification of surfactant solubilized membrane proteins synthesized in recombinant and cell free expression systems. Elastin-like polypeptide (ELP) tags, which have been designed to allow non-chromatographic purification of soluble proteins, offer a potential means to enable facile large-scale purification of detergent solubilized recombinant membrane proteins. However, the phase transition temperature (T_t) of ELPs is sensitive

to the addition of cosolutes and many detergents increase the T_t of ELPs to temperatures greater than the thermal denaturation temperature of many proteins that are fused to the ELP, hence prohibiting their use for protein purification. To identify detergents that would satisfy the dual and potentially conflicting requirements of stabilizing membrane proteins fused to an ELP, we screened different detergents with respect to their effect on the T_t of ELP[$V_5A_2G_3$-180]. We found that dodecyl maltoside (DDM), a detergent that is commonly used to solubilize recombinantly expressed membrane proteins, did not significantly alter the phase transition characteristics of ELPs or their structure as probed by a temperature-programmed turbidity assay and circular dichroism spectroscopy. Our results clearly indicate that DDM does not affect the inverse transition cycling of ELPs and therefore may be useful to purify membrane proteins which are otherwise difficult to extract and purify by affinity chromatography.

BI-TuP8 Self-assembly of Proteins on Well-defined Sapphire Surfaces*, **T. Isono**, K. Yamazaki, T. Ogino*, Yokohama National University, Japan

Non-specific adsorption is a very serious issue when biomolecules are immobilized on solid surfaces. Non-specific adsorption should be suppressed especially in application of solid surfaces to biosensors or implant devices. We have tried to control protein adsorption on step-controlled sapphire surfaces. Because sapphire surface is chemically stable in liquid as well as in air, it is a suitable material for bioapplication. To control interactions between the sapphire surfaces and protein molecules, we designed surface atomic structures. In this study, we report on self-assembly of several kinds of protein molecules by using well-defined sapphire surfaces. Single crystalline sapphire (0001) surfaces were used for protein adsorption. By a high temperature annealing, the sapphire surfaces are covered with bunched steps accompanied with crossing steps when the miscut direction slightly tilts from the stable direction of atomic steps. These surfaces were cleaned by a mixture of sulfuric acid and hydrogen peroxide. We call this surface oxidized surface. Two domains, which exhibit different hydrophilicity and charge density from each other, coexist on this surface. Center regions of the terraces (domain A) are relatively hydrophobic and weakly charged, and the others (domain B) hydrophilic and negatively charged. To control the surface chemical properties, the oxidized surfaces were slightly etched by phosphoric acid. We call this surface etched surface. Protein molecules were physically adsorbed on the oxidized and the etched surfaces in a buffer solution. Protein adsorption patterns were observed by atomic force microscopy in the same buffer solution. When the oxidized surfaces were immersed in a solution of ferritin molecules whose surfaces exhibit a negative charge, ferritin molecules were selectively adsorbed on the domain A by the electrical repulsion between the domain B and the molecules. On the other hand, avidin molecules whose surfaces exhibit a positive charge were selectively adsorbed on the domain B by the attractive interaction. These protein molecules were adsorbed on the sapphire surfaces depending on the surface charges. However, small amount of avidin molecules were adsorbed on the hydrophobic domain A. Generally, it is easier for protein molecules to be adsorbed on hydrophobic surfaces than hydrophilic ones. To make the domain A hydrophilic, the oxidized surfaces were etched using phosphoric acid. When the etched surfaces were immersed in a solution of avidin molecules, avidin molecules were selectively adsorbed without non-specific adsorption on the domain A.

BI-TuP10 Lubricin Prevents Degenerative Changes in Articular Cartilage Structure and Morphology*, **J. Coles**,* Duke University*, C. Cha,* Brown University*, M. Warman,* Boston Children's Hospital*, G. Jay,* Brown University*, F. Guilak, S. Zauscher*, Duke University

Lubricin is a mucin-like glycoprotein which contributes to boundary lubrication in joints and is also thought to have a role in protecting cartilage surfaces. Direct studies of joint protection by lubricin have been difficult but a lubricin null mouse has been developed recently, providing completely lubricin-free cartilage for study. We have shown that atomic force microscopy can be used for measurements of interfacial friction in the boundary lubrication regime and use this technique to measure friction directly on cartilage not expressing lubricin. We further use atomic force microscopy and histology to characterize stiffness and surface and subsurface morphology of these joints. While friction measured directly on lubricin null cartilage was only slightly lower than on wild type cartilage, surface structure and mechanical integrity were altered significantly. Lubricin null cartilage surfaces were significantly rougher, stiffness did not develop normally, and glycosaminoglycan (a core structural component of cartilage) concentration near cells was lost as joints developed. While reduction of friction is likely an important factor in lubricin's role in protecting cartilage, our measurements on lubricin null cartilage suggest that lubricin may additionally protect cartilage through other mechanisms.

BI-TuP11 Plasma Polymerization Induced Structural Modification of PCL*, **A.M. Sandstrom**, L. Grøndahl, J.J. Cooper-White*, University of Queensland, Australia

Cellular response to tissue culture scaffolds is thought to be directed by chemical and topographical cues from surfaces which bind biological motifs recognized by cell receptors. Plasma polymerization (PP) is frequently used to functionalize surfaces for improved biocompatibility. It has been reported that the type and distribution of functional groups created on a surface is dependent on plasma parameters. However, in addition to changing chemistry, modification of plasma parameters is also likely to introduce topographical variation to the sample. The aim of this work was to elucidate the combined effects of plasma power and treatment time on the surface chemistry and topographical features of the substrate.

Scaffolds [5% poly(ε-caprolactone) (PCL)/1,4-dioxane] were prepared using thermally induced phase separation. Samples were plasma polymerized in a custom-built radio frequency reactor using heptylamine (HA) at 20W and 50W for 30s and 60s. Additional substrates were prepared by spincoating PCL on Si wafers. For each of the four treatment conditions, surface homogeneity was confirmed across the wafers and scaffold sections via XPS. Surface topography was investigated on wafers by AFM, and scaffolds were examined using SEM.

Disappearance of the PCL carboxyl (C=O) peak following PP for all treatment conditions suggests complete coverage (>10nm) of the top surface of all substrates by PPHA. The N/C ratio was slightly higher on the Si wafer than the scaffold. Treatment was homogenous across individual samples. Slight chemical functionality shifts were found between samples, which may reflect intrinsic plasma differences or post-PP oxidative variation.

Polymer aggregates formed on Si following PPHA treatment at 20W, but were diminished at 50W. Topographical changes were more pronounced on PPHA PCL-coated Si. Although spherulite size variation was minimal between untreated semi-crystalline PCL and PPHA PCL on Si for most treatments, complete recrystallization of the base polymer was observed at 50W 60s. Change in surface roughness was evidenced by disappearance of well-defined fibrous domains as treatment power and time increase for all samples, except at 50W 60s when distinct spherulites re-emerged.

Highly reactive HA recombination led to aggregate size reduction on Si, whereas PPHA on PCL appeared to affect structural organization of the substrate. Scaffold morphology also changed following PPHA, as more energetically favored fibrous extensions in the porous region of the surface were observed by SEM. These results may be used in conjunction with cellular studies to tune PPHA reactions on scaffolds as required for support of various cell types.

BI-TuP12 Molecular Dynamics Simulation of Interactions between Structured Peptides and Functionalized Solid Surfaces*, **G. Collier**, R.A. Latour, S.J. Stuart*, Clemson University

The chemical and physical interactions between proteins and biomaterial surfaces govern the biocompatibility of those materials when introduced into a living system. Therefore, the possibility of controlling biocompatibility on a molecular level through the strategic design of biomaterials begins with the study of these interactions at the atomic level. Without an accurate molecular description of the way that proteins interact with biomaterials, biomaterial design is primarily relegated to a trial-and-error approach. To address this issue, we have begun to evaluate the applicability of existing all-atom molecular simulation methods and force fields to the unique situation of simulating the interaction of structured peptides with solid biomaterial surfaces.

Today's most advanced molecular modeling tools do not include parameterization options for molecular systems interacting with solid biomaterial surfaces, so we have undertaken a variety of pilot studies to guide the development of our methods for use in more complex simulations. These pilot studies include nanosecond-scale simulations of structured peptides interacting with charged and uncharged self-assembled monolayer (SAM) surfaces, ion distributions over a charged SAM surface, peptide-peptide interaction studies, peptide stability studies, analysis of environmental changes resulting from peptide conformation changes, and analysis of the role of water molecules in the peptide-surface adsorption process. The adsorption and stability studies are being conducted using multiple molecular modeling force fields, enabling a comparison of their performance and usefulness in the simulation of these unique systems. The majority of this work has been conducted using replica-exchange molecular dynamics (REMD) techniques in order to optimize conformational sampling, resulting in the most efficient path toward structural data that can be compared with experimental results.

The results from these studies are enabling us to assess the suitability of currently available molecular simulation methods and force fields and are providing us with a better understanding of peptide-surface interactions at the atomic level.

BI-TuP13 ToF-SIMS Study of Fibronectin Orientation on Self-Assembled Monolayers, ***L. Árnadóttir***, *J. Brison, L.J. Gamble*, University of Washington

Protein adsorption and orientation plays a critical role in many biomedical applications. 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. In this study the adsorbed orientation and conformation of the 7-10 fragment of FN ($FNIII_{7-10}$) was studied on four different model surfaces (self-assembled monolayers (SAM) of C_{11} alkanethiols on Au, $-CH_3$, $-NH_2$, -COOH and -OH terminated SAM). The $FNIII_{7-10}$ fragment incorporates both the Arg-Gly-Asp (RGD) FN receptor binding motif and the PHSRN synergy site which participate in the RGD binding. The effect of different surface chemistries on binding and adsorption configuration was investigated using X-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectrometry (ToF-SIMS). XPS showed significantly higher protein coverage on the hydrophobic $-CH_3$ terminated surface than the hydrophilic and negatively charged –COOH terminated surface. Both XPS data and SIMS peak intensities for certain amino acid were used to verify the adsorption process and monolayer coverage. Full coverage was determined by a plateau in SIMS peak intensities with increasing protein exposure. A principal component analysis (PCA) of the ToF-SIMS data on surfaces with similar sub-monolayer protein coverage was then used to determine changes in the spectra that differed when the fragment was adsorbed on various surfaces. These results are related to different orientation or conformations of the fragment on the different surfaces.

BI-TuP14 Wear-Rate Behavior of Hydroxyapatite-Coated Titanium, ***A.F. Jankowski***, Texas Tech University

Calcium phosphate-based ceramics are used in medicine and dentistry due to excellent biocompatibility with human tissues. The formation of hydroxyapatite coatings with a porosity gradient on the surface of metal implants can provide a surface suitable for the in growth of connective tissue. A need exists to both protect the metal substrate against corrosion and provide sufficient mechanical adhesion. Hydroxyapatite coatings have been produced by the reactive, radio-frequency sputtering of stoichiometric $Ca_{10}(PO_4)_6(OH)_2$ targets using planar magnetrons onto titanium-coated silicon substrates. A fully dense and thin initial-coating layer appears to avoid cracking, improves substrate adhesion, and provides corrosion protection (J. Mater. Res. 16, 2001, 3238). The effect of varying the wear rate is now evaluated on the mechanical behavior of sputter deposited hydroxyapatite coatings.

BI-TuP15 Asymmetric Hybridization Behavior Exhibited by DNA Probes Containing Surface-Attachment Ligands and Self-Complementary Sequences, *S.M. Schreiner, D.F. Shudy,* University of Wisconsin, ***D.Y. Petrovykh,*** Naval Research Laboratory and University of Maryland, College Park, *A. Opdahl*, University of Wisconsin

We demonstrate the impact that intra-molecular and nucleotide-gold interactions have on conformation of surface-immobilized DNA probes and their hybridization behavior. We take advantage of a method based on the intrinsic affinity of adenine nucleotides for gold (Opdahl et al., PNAS, **104**, 9–14, 2007) to immobilize block-oligonucleotides having sequences that follow a $d(A_k\text{-}T_m\text{-}N_n)$ pattern: a block of k adenines [$d(A_k)$], followed by a block of m thymines [$d(T_m)$], and a block of n (arbitrary) nucleotides [$d(N_n)$]. Such block-oligos assemble on gold via the d(A) blocks, which allow a high degree of control over DNA surface coverage and conformation. We characterize immobilization and hybridization of these DNA probes using x-ray photoelectron spectroscopy (XPS) and surface plasmon resonance (SPR) imaging, specifically to compare two 15-nucleotide N_n sequences: 15 thymines (T15) and a realistic arbitrary sequence (P15). For A15-T5-P15 probes, the hybridization efficiency at the P15 end is enhanced 2-fold compared to that at the A15 end. For A15-T20, which forms a hairpin in solution, we observe a larger asymmetry of hybridization efficiencies when immobilized on gold: while the T15 end is highly accessible for hybridization, the A15 end has virtually no hybridization activity. A thiolated version of the same probe (A15-T20-SH), in contrast, exhibits little asymmetry and overall low hybridization efficiency with either A15 or T15, consistent with an immobilized structure of a stable hairpin. Additional experiments, whereby a displacement thiol is added to reduce DNA-gold interactions, are used to support our inferences about the role played by intra-molecular and surface interactions in immobilization and hybridization of DNA probes.

Biomaterial Interfaces
Room: K - Session BI+AS+BM+MS-WeM

Array-Based Sensors and Diagnostics: Grand Challenges
Moderator: D.W. Grainger, University of Utah, J. Shumaker-Parry, University of Utah

8:00am **BI+AS+BM+MS-WeM1 Design of Antibody Array-Based Sensors for Disease Proteomics: Grand Challenges**, ***C. Wingren***, Lund University, Sweden **INVITED**

Antibody-based microarray is a new proteomic methodology setting a novel standard for analysing complex, non-fractionated proteomes. The first generation of antibody micro- and nanoarrays has already demonstrated its potential for generating detailed protein expression profiles, or protein maps, of human body fluids in health and disease, paving the way for new discoveries within the field of disease proteomics. The process of designing highly miniaturized, high-density and high-performing antibody array set-ups have, however, proven to be challenging. In this presentation, the key technological challenges that must be resolved in a cross-disciplinary manner before true global proteome analysis can be performed using antibody array-based sensors will be presented and discussed.

In this context, we have successfully designed a set of state-of-the-art recombinant antibody array technology platforms for high-throughput proteomics. In more detail, we use human recombinant single-chain Fv (scFv) antibody fragments, microarray adapted by molecular design as probes, displaying an outstanding on-chip functionality and stability. Uniquely, the platforms allows us to target both water-soluble as well as membrane proteins in a highly multiplexed and sensitive (pM to fM range) manner in complete, i.e. non-fractionated, directly labeled complex proteomes. Platforms compatible with a wide range of proteomes, including serum, plasma, urine, cell lysates, tissue extracts, intact cells etc, have been successfully designed. In addition, the first steps towards implementing label-free sensing (MS, MS -MS and SPRi) as well as designing self-addressable microarrays and miniaturized attovial-based nanoarrays as well as planar nanoarrays have been taken, clearly expanding the repertoire of technology platforms. The applicability of the platform(s) for differential high-content screening of clinical samples has been validated in a set of key applications within the field of oncoproteomics, autoimmunity, inflammatory diseases and allergy. The optimized antibody microarray technology platforms, as well as data from the screening analysis will be presented in context of the grand challenges the field experiences.

8:40am **BI+AS+BM+MS-WeM3 Development, Validation and Application of Q-Plex Array Technology**, ***M. Groll***, Quansys Biosciences Quansys Biosciences **INVITED**

The Quansys Q-Plex (multiplex ELISA) Array is a fully quantitative ELISA-based test where up to 25 distinct capture antibodies have been absorbed to each well of a 96-well plate in a defined array. This array is composed of 20 nanoliter spots with 350µm diameters and a pitch of 650µm between spots. Each spot represents a different distinct capture antibody population.

Using less than 30 µl of sample, up to 84 different samples can be assayed for all 25 unique analytes in less than 2.5 hours. Sensitivity is system dependent and typically ranges between 30 pg/ml to less than 1 pg/ml. All of the antibodies used in the Q-Plex arrays have been subject to a rigorous and comprehensive cross reactivity protocol and verified to be non-cross reactive with any other system on the array. Detection of this array is performed using the Quansys Q-View Imaging System. The image is then auto-processed using Quansys Q-View Software and concentrations for each analyte are output for the sample.

9:20am **BI+AS+BM+MS-WeM5 Drop on Demand Ink Jet Methods for Development and Manufacturing of Array Based Sensors and Diagnostics**, ***T.C. Tisone***, *A.V. Lemmo*, BioDot Inc.

The development and manufacturing of array based formats requires the transfer of biomarker reagents to a carrier substrate which forms the basis of a sensor for executing a multiplexed assay for research and diagnostics applications. The typical volume range for these types of assays is in the range of 100 pL up to 1000 nL: which lies in the range of commercial drop on demand piezoelectric and solenoid drop on demand dispensers. This presentation will discuss aspects of the physics and chemistry of successful applications of drop on demand methods to provide quantitative and high throughput reagent transfer to sensor substrates suitable for both Development and Manufacturing. Issues of drop formation, drop/substrate interactions and reagent/substrate interactions will be discussed. The agenda is to understand what role dispensing plays in the assay function.

10:40am **BI+AS+BM+MS-WeM9 New Molecular Strategies to Suppress Noise and Amplify Signal in Protein and DNA Microarrays**, ***A. Chilkoti***, Duke University **INVITED**

This talk will highlight recent work from my laboratory that addresses new interfacial technologies to suppress noise (N) and amplify signal (S) leading to heterogeneous assays with extraordinarily high S/N. In the first demonstration, I will focus on the adventitious adsorption of proteins as the primary factor that controls the limit-of-detection (LOD) of protein microarrays and limits the measurement of analytes from complex mixtures such as serum or blood. I will show data on a new protein microarray assay where background adsorption is effectively eliminated through the use of a protein-resistant –nonfouling– polymer brush. These “zero background” protein microarrays were successfully used to quantify protein analytes in serum with femtomolar LOD and a dynamic range of six orders of magnitude of analyte concentration. These LODs are 100-fold lower when compared to the same protein microarrays spotted on a conventional polymer substrate that displays high binding capacity but significant adventitious protein adsorption. This study also provided the first demonstration of the interrogation of an analyte directly from undiluted, whole blood by a protein microarray with a LOD of ~15 fM. Next, I will summarize recent work in my laboratory on the development of a new isothermal fluorescence signal amplification and detection scheme that exploits the ability of terminal deoxynucleotidyl transferase (TdTase) to add up to 100 fluorescent nucleotides to the end of a short DNA tag with an exposed 3’-OH. I will show how DNA microarrays that are printed on the nonfouling polymer brush exhibit low background signal, yet allow on-chip fluorescence signal amplification, leading to DNA microarrays that exhibit a sub-picomolar LOD, which appears to be the lowest LOD reported for DNA microarrays, to date.

11:20am **BI+AS+BM+MS-WeM11 SwitchDNA Biosensors for the Label-Free Detection and Sizing of Protein Targets on a Chip**, ***U. Rant***, *W. Kaiser, J. Knezevic, E. Pringsheim, M. Maruyama, P. Hampel,* Technische Universitaet Munich, Germany, *K. Arinaga,* Fujitsu Laboratories Ltd., Japan, *G. Abstreiter*, Technische Universitaet Munich, Germany

We introduce a chip-compatible scheme for the label-free detection of proteins in real-time that is based on the electrically driven conformation-switching of DNA oligonucleotides on metal surfaces. The switching behavior is a sensitive indicator for the specific recognition of IgG antibodies and antibody-fragments, which can be detected in quantities of less than 1 amol on the sensor surface. Moreover, we show how the dynamics of the induced molecular motion can be monitored by measuring the high-frequency switching response as well as by time-resolved fluorescence measurements. When proteins bind to the layer, the increase in hydrodynamic drag slows the switching dynamics, which allows us to determine the size of the captured proteins. We demonstrate the identification of different antibody fragments by means of their kinetic fingerprint. The switchDNA method represents a generic approach to simultaneously detect and size target molecules using a single analytical platform.

11:40am **BI+AS+BM+MS-WeM12 Nanomechanical Readout of DNA Microarrays**, *S. Husale,* Rowland Institute at Harvard University, ***H.H.J. Persson***, Stanford University, *O. Sahin*, Rowland Institute at Harvard University

DNA microarrays have enabled high throughput analysis of gene-expression and genotyping. However, they still suffer from limited dynamic range and rely heavily on enzymatic manipulations and amplification to create detectable signals. Here we present application of a novel nanomechanical detection method to microarray analysis that may circumvent these disadvantages. It is based upon a modified atomic force microscope (AFM) that can map mechanical properties of surfaces at high speed and spatial resolution. Mechanical measurements can reliably discriminate single and double stranded DNA on a surface. Automated image analysis reveals hybridized molecules with single molecule precision, thus providing a digital measure of hybridization. This method can detect a broad range of target concentrations with a limit of detection in the low attomolar concentration range without any labeling, enzymatic manipulations, and amplification. We demonstrate the performance of this technique by measuring differential expressions of miRNAs in tumor samples, which has been shown to help discriminate tissue origins of metastatic tumors.

Biomaterial Interfaces
Room: K - Session BI+AS+NS-WeA

Quantitative Nanoscale Sensing at Biosurfaces and Interfaces
Moderator: F. Höök, Chalmers University of Technology, P. Kingshott, Aarhus University, Denmark

2:00pm **BI+AS+NS-WeA1 Characterizing Self-Assembled Supported Lipid Membranes for Biosensing**, ***E.O. Reimhult***, ETH Zurich, Switzerland **INVITED**

More than 50% of all drug targets are membrane proteins, which require a lipid membrane environment to retain correct conformation and function. This highlights the need to create sensing tools for analytical profiling of transmembrane protein function subject to e.g. drug binding. Furthermore, it is increasingly realized that the compositionally complex and dynamically rearranging lipid membranes can be important active regulators of biological function in their own right. The complexity of the *in vivo* cell membrane and the need to apply high throughput techniques like arrays and highly surface sensitive analytical techniques make model systems highly desirable. Thus, supported lipid bilayers (SLBs) which combine control of membrane properties with surface analytical techniques receive increasing interest.

Biosensor interfaces can be easily functionalized with an SLB by self-assembly from liposomes. However, design of more native-like SLBs, e.g., having diverse lipid compositions, including glycolipids or mimics thereof, demands further developments of this assembly technique. This in turn prompts for more advanced characterization of the formation and structure of SLBs.

We present advances in instrumentation and interpretation of data from multi-technique studies of liposome adsorption and SLB formation, which enhance the understanding of the assembly process and the sensor response obtained for different membrane conformations. In particular, we demonstrate advances in waveguide spectroscopy which allow for characterization of the rupture kinetics of supported lipid bilayers by liposome fusion, but also to in real time distinguish differences in structure for membranes of different compositions and under various environmental conditions. These advances also open the possibility to study differential binding to and into SLBs and to use rearrangements in the SLB as an amplifier of membrane protein binding events.

As examples, we also present the results of such detailed multi-technique characterization of the self-assembly of new supported lipid membrane mimics, e.g., bacterial membrane mimics containing lipopolysaccharides and poly(ethylene glycol)-lipids, including how the presence of a polymer directly attached to the lipids affects the self-assembly and how the hydrophilic polymer is distributed and rearranged in the membrane under mechanical perturbation. Such self-assembled polymer-membranes have great potential for creation of membrane arrays incorporating membrane proteins thanks to high stability and less perturbation of the membrane components due to the mobile polymer spacer layer.

2:40pm **BI+AS+NS-WeA3 Nanoplasmonic Biosensing: Artificial Cell Membranes, Structural Changes and Quantification of Bound Mass**, ***M. Jonsson***, *A. Dahlin, P. Jönsson, S. Petronis, F. Höök*, Chalmers University of Technology, Sweden

The resonance condition for excitation of plasmons associated with metal nanostructures is highly sensitive to changes in the interfacial refractive index, which has made the phenomenon highly popular as transducer principle for label-free sensing of biomolecular recognition reactions. There is a particular need for sensor concepts that are compatible with studies of the cell membrane, which can be explained from the fact that more than half of the most commonly used drugs are directed towards membrane-associated reactions. This is also relevant with respect to diagnostics of viral diseases, because viruses typically infect host cells via adsorption to the cell membrane. During the past years we have developed nanoplasmonic biosensing platforms that are compatible with studies of artificial cell membranes, such as lipid vesicles and supported lipid bilayers (SLBs).[1-3] In addition to probing specific binding of ligands to membrane receptors, we showed that nanoplasmonic sensors provide a unique means to probe biomolecular structural changes, such as during the formation of a SLB from adsorption and rupture of lipid vesicles.[1]

We have previously used a metal film perforated with nanoholes as an electrode for combined nanoplasmonic and quartz crystal microbalance measurements.[3,4] Besides two independent measures on biomolecular structural changes, the combined sensor setup was shown to provide new information that enabled the quantification of adsorbed mass on the sensor surface with only the density of the molecules as unknown parameter.[3]

In the current work we utilize the continuity of a perforated plasmon active metal film to fabricate nanoplasmonic pores with liquid access to both sides of the nanopores.[5] This structure opens up for a wide range of novel applications. For example, extending our previous work on plasmonics and cell membrane mimics, an appealing possibility is to measure transport of both charged and non-charged molecules through lipid membranes that span the pores. Plasmonic pores can also be used for flow-through sensing, where flowing the target molecules through the pores will facilitate molecules to reach the sensor surface in an efficient way and circumvent limitations due to mass-transport.[6]

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3:00pm **BI+AS+NS-WeA4 Transfer of Biomolecules between Lipid Membranes**, ***A. Kunze***, *S. Svedhem,* Chalmers University of Technology, Sweden, *P. Sjövall,* SP Technical Research Institute of Sweden, *B.H. Kasemo*, Chalmers University of Technology, Sweden

The study of the interaction between biomembranes is of great interest for both basic research and applications in biosensing technology. In biological systems the interaction between membranes including transfer of biomolecules plays a pivotal role. For instance, it is central in energy supply to and communication between cells and for the function of a large number of drugs. A controlled transfer of lipid molecules, or other biomolecules, between lipid vesicles (liposomes) and solid supported lipid bilayers (SLBs) provides a new platform for modifying and controlling SLBs that can be used in biosensing technology. Mechanistic studies of this process are furthermore important for the understanding of a number of important biomolecule-membrane and inter-membrane events.

We will present how transfer of biomolecules between an SLB and liposomes can be monitored in real-time giving more insight into the complex mechanism of transfer including influence of electrostatic interaction, ionic strength, phase and molecular structure of lipids, as well as time scale of the transfer process. Recent results show that the interaction process consists of an attachment-transfer-detachment (ATD) sequence, where added liposomes first attach to a preformed SLB, then transfer lipid molecules and eventually detach, leaving behind a compositionally modified SLB and ditto vesicles.[1] We will demonstrate how the ATD process can be used for *in situ* modifications, changing the membrane composition, e.g. for the formation of a highly stabilized (SDS-resistant) lipid monolayer on TiO_2, which can then be used for the reassembly of an SLB.[2] We propose this as a promising method for *in situ* preparation of asymmetric SLBs.

The main experimental techniques used to study these processes at these interfaces between two biomembranes are the quartz crystal microbalance with dissipation monitoring (QCM-D), t otal internal reflection fluorescence microscopy (TIRF), fluorescence microscopy and time-of-flight secondary ion mass spectrometry (TOF-SIMS) and optical reflectometry.

[1] Kunze, A.; Svedhem, S.; Kasemo, B. Lipid Transfer between Charged Supported Lipid Bilayers and Oppositely charged Vesicles, Langmuir in press

[2] Kunze, A.; Sjövall, P., Kasemo, B.; Svedhem, S. In situ preparation and modification of supported lipid layers by lipid transfer from vesicles studied by QCM-D and TOF-SIMS, J. Am. Chem. Soc., 131:2450-2451, 2009

4:00pm **BI+AS+NS-WeA7 Nanopores for Sensing Membrane Processes and Enzyme Reactions**, ***M. Mayer***, University of Michigan **INVITED**

This talk demonstrates that pores with diameters below 50 nanometers make it possible to detect enzyme reactions, molecular phases transitions, and nanoscale self-assemblies *in situ* and in real time. For instance, coating the inner walls of nanopores with self-assembled lipid bilayers, afforded controlled shrinkage of this pore to a size that made it possible to detect individual proteins. Remarkably, the extent of pore shrinkage could be

controlled with sub-nanometer precision by the chain lengths of the acyl chains on the lipids that were chosen to assemble the bilayer. Due to the extreme sensitivity of single-channel recording of ion currents through nanopores, this approach made it possible to monitor molecular changes and rearrangements of the lipid bilayer. These changes included phase transitions, variations in membrane composition, and enzymatic reactions on membranes. For example, this approach made it possible to monitor the activity of attomolar amounts of phospholipase D (PLD) and phospholipase C (PLC) – two membrane-active enzymes that are critical for cell signaling.

4:40pm **BI+AS+NS-WeA9 Development of Microresonator Arrrays for Mass and Viscoelastic Characterization of Adsorbed Molecular and Biomolecular Thin Films**, ***D.L. Allara***, *S. Tadidagapa, P. Kao*, Pennsylvania State University

A multiple pixel micromachined quartz crystal resonator array with a fundamental resonance frequency in the 60-100 MHz range has been designed, fabricated, and tested for applications to accurate mass and viscoelastic measurements of adsorbed thin molecular and biomolecular films. Operating with high Q-factors in the range of 25000–50000 and appropriately lower in liquids, the high stability and inherent low noise of the quartz crystals allow for an unprecedented resolution of one part in 10 million for density/viscosity variations. Further, multiple pixels, capable of independent functionalization with SAMs, can be tracked in parallel to give large numbers of independent measurements simultaneously. By measuring the frequency decrease at overtone frequencies it also is possible to vary the decay length of the shear wave away from the electrode and thereby identify individual variations in the density and viscosity of the local environment and accurately monitor small changes in the viscoelastic loading of adsorbed films. The performance of the resonator is illustrated with examples such as the adsorbed protein films in which the damping factor undergoes an order of magnitude change in transitioning from monolayer to multilayer adsorption. This aspect is highly desirable for accurate determination of behavior such as conformational changes.

5:00pm **BI+AS+NS-WeA10 Plasmonically Coupled Nanoparticle-Film Molecular Ruler**, ***R.T. Hill***, *J.J. Mock, A. Degiron, S. Zauscher, D.R. Smith, A. Chilkoti*, Duke University

Experimental analysis of the plasmonic scattering properties of gold nanoparticles controllably placed nanometers away from a gold metal film shows that the spectral response of this system results from the interplay between the localized plasmon resonance of the nanoparticle and the surface plasmon polaritons of the gold film, as previously predicted by theoretical studies. In addition, the metal film induces a polarization to the single nanoparticle light scattering resulting in a doughnut-shaped point spread function when imaged in the far-field. Both the spectral response and the polarization effects are highly sensitive to the nanoparticle-film separation distance, and thus, the plasmonically coupled NP-Film system represents a new variant of the previously reported plasmonic molecular rulers. A surface-based molecular ruler shows promise in potential biosensor and diagnostic devices.

5:20pm **BI+AS+NS-WeA11 Label-free Imaging of Cell Adhesion Dynamics using Surface Plasmon Resonance Imaging Ellipsometry**, ***D.W. Moon***, *J. Gil, W. CheGal, H. Cho, S. Kim*, Korea Research Institute of Standards and Science, S. Korea

The interaction between cell and extracellular matrix (ECM) governs multiple cellular functions and contributes to promote inflammation and tumor metastasis. Therefore, cellular behavior needs to be monitored in the ECM interactive circumstance. Most of previous studies on cell adhesion are based on immunofluorescence microscopy. For cell adhesion dynamics studies, label-free optical techniques that can monitor continuously cell-ECM interfaces for living cells are required.

Here we developed surface plasmon resonance imaging ellipsometry (SPRIE) which can simply image cell-ECM interfaces for live cells with high contrast and at real-time. To visualize cell adhesions to ECM, null-type imaging ellipsometry technique with the attenuated total reflection coupler was applied and both of transverse magnetic and electric waves were made use of. These characteristics make it possible to acquire the high contrast image of cell adhesions. Different features and dynamics of cell adhesion patters in ~ 100 nm cell-ECM interfaces were observed for A10, human coronary artery smooth muscle cell hCASMC, and human umbilical vein endothelial cells (HUVEC) on fibronectin and collagen ECM layers with 1 µm spatial resolution and 30 sec time interval upto 3 days. Harmonized changes of entire adhesion proteins were observed during cell division and cell migration through our imaging system without any labeling. SPRIE images were compared with confocal fluorescence microscopic images of cell adhesion proteins for validation of SPRIE images. Preliminary results on SPRIE studies on the effect of shear force on cell adhesion and migration will be also discussed.

We expect that SPRIE cell adhesion dynamic imaging methods would be useful for further understanding of cell biology and development of drug screening methodology relevant to cell adhesion and migration.

5:40pm **BI+AS+NS-WeA12 Label-Free Determination of Protein-Ligand Equilibrium in Aqueous Solution using Overlayer Enhanced Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (OE-ATR-FTIR)**, ***T.C. Ruthenburg***, *S.S.N. Park, T.A. Aweda, C.F. Meares, D.P. Land*, University of California, Davis

Protein binding/affinity studies are often performed using Surface Plasmon Resonance techniques that don't produce much spectral information. Measurement of protein binding affinity using FTIR is traditionally performed using high protein concentration or deuterated solvent. By immobilizing a protein near the surface of a gold-coated germanium internal reflection element interactions can be measured between an immobilized protein and small molecules in aqueous solution. Using flow injection analysis the on and off rates of these interactions and dissociation constant for the system can be determined. The dissociation constant for the molecule Yttrium-aminobenzyl-DOTA binding to the antibody 2D12.5 system was determined.

Applied Surface Science
Room: K - Session AS2+BI-ThM

Scanning Probe Studies of Biological Materials
Moderator: I.S. Gilmore, National Physical Laboratory, UK, S. Zauscher, Duke University

8:00am **AS2+BI-ThM1 Beyond the Optical Resolution in Living Cell: Biomedical Applications of Scanning Ion Conductance Microscopy**, *Y.E. Korchev,* Imperial College London, UK, ***S. Allen***, The University of Nottingham, UK **INVITED**

Molecular Biology has advanced we know much about the individual molecular components that make up living cells down to the level of the individual atoms. The challenge, however, is to fully understand the functional integration of these components. This requires determining how the molecular machines that make up a living cell are organized and interact together not at the atomic length scale but on a nm scale. To do this we need to develop and applying nanoscale techniques for the visualisation and quantification of cell machinery in real-time and on living cells. This will lead to detailed, quantitative models of sub-cellular structures and molecular complexes under different conditions for both normal and diseased cells. This approach ultimately requires the development of novel biophysical methods. We have recently pioneered the development of an array of new and powerful biophysical tools based on Scanning Ion Conductance Microscopy that allow quantitative measurements and non-invasive functional imaging of single protein molecules in living cells. Scanning ion conductance microscopy and a battery of associated innovative methods are unique among current imaging techniques, not only in spatial resolution of living and functioning cells, but also in the rich combination of imaging with other functional and dynamical interrogation methods. These methods, crucially, will facilitate the study of integrated nano-behaviour in living cells in health and disease.

8:40am **AS2+BI-ThM3 Nanometer Scale Patterning of Biomolecules using Near-Field Optical Methods**, ***G.J. Leggett***, University of Sheffield, UK

The integration of top-down (lithographic) and bottom-up (synthetic) methods remains one of the outstanding challenges in molecular nanoscience. There are no established tools for the manipulation of surface chemical structure in the length range from ca. 100 nm to the dimensions of a single biomacromolecule. Scanning near-field photolithography (SNP), in which a scanning near-field optical microscope coupled to a UV laser is employed as a light source, may be used to execute specific molecular transformations with a spatial resolution of a few tens of nm and, at best, 9 nm (ca. $\lambda/30$). Several strategies will be described for the patterning of proteins and nucleic acids on metal and oxide surfaces. We demonstrate two approaches. First, photodegradation of oligo(ethylene glycol) (OEG) functionalised surfaces provides a simple route to the covalent attachment of proteins to non-fouling surfaces. Photodegradation of OEG-terminated thiols provides a simple, one-step route to the conversion of a protein-resistant surface to a protein-binding one. Similar methodologies may be extended to oxide surfaces, through the formation of OEG-functionalised siloxane films. Photodegradation yields aldehyde functionalities, to which nitrilo triacetic acid (NTA) functionalised amines may be coupled, and subsequently dertivatised, following nickel complexation, with histidine-tagged proteins, providing a facile route to the site-specific immobilisation of proteins on glass. Second, siloxane monolayers offer fruitful opportunities for the incorporation of synthetic chemical methods into nanolithography. Halogenated monolayers may be converted to aldehydes or to carboxylic acids, by controlling the exposure. Alternatively, 2-nitrophenylpropyloxycarbonyl (NPPOC) protected aminosiloxane monolayers on glass may be selectively deprotected by SNP, yielding amine groups for further functionalisation with very high spatial resolution. The synthetic flexibility and versatility of photochemical methods, when combined with near-field methods for control of exposure, offers enormous potential for integrating top-down and bottom-up methods.

9:00am **AS2+BI-ThM4 Nanomechanics of Glycopeptide Resistant Superbugs and Superdrugs**, ***J. Ndieyira,*** Uni. College London, UK and Jomo Kenyatta Univ. of Agriculture and Tech., Kenya, *A. Donoso Barrera, M. Vogtli,* Uni. College London, UK, *M. Sushko,* Uni. College London, UK and PNNL, *D. Zhou,* Univ. of Leeds, UK, *M. Cooper,* The Univ. of Queensland, Australia, *C. Abell,* Univ. of Cambridge, UK, *T. Strunz, G. Aeppli, R. McKendry*, Uni. College London, UK

The discovery of penicillin in 1928 marked the beginning of a remarkable new era of antibiotic ‘wonder drugs’, saving millions of lives across the world. However the widespread and often indiscriminate use of antibiotics has fuelled the alarming growth of antibiotic resistant superbugs, including methicillin resistant Staphylococcus aureus (MRSA) and vancomycin resistant Enterococci (VRE). To remain one step ahead of the superbugs, there is now an urgent need to develop new antibiotics and yet the drug pipeline is severely limited. We recently reported the nanomechanical detection of vancomycin-cell wall peptide interactions on cantilever arrays and discriminated between vancomycin-sensitive and vancomycin-resistant phenotypes.[1] In this talk I will present our new work which exploits this technology for the search of new superdrugs active against VRE. We have investigated a series of vancomycin derivatives and detect a dramatic enhancement in surface binding affinities compared to homogeneous solution measurements. We identify a glycopeptide which binds 11,000 more strongly to resistant peptide analogues. Our findings reveal fundamental new insights into the mechanism of antibiotic bindings to a model bacterial cell wall peptides using nanosensors, which not only has important implications on the design of new devices with significantly improved antibiotic detection sensitivity but will also impact on our understanding of the mode of action of antibiotics on intact bacteria. These findings highlight the potential of BioMEMs devices for application in pharmaceutical industry and will accelerate the discovery of new antibiotics.

[1] ‘Nanomechanical detection of antibiotic mucopeptide binding in a model for superbug drug resistance.’ Ndieyira, W.N, Watari, M., Donoso-Barrera, A., Batchelor, M., Zhou, D., Vogtli, M., Bactchelor, M., Cooper, M., Strunz, T., Abell, C.A., Rayment, T., Aeppli, G. & McKendry R.A. *Nature Nanotechnology* **3**, 691 - 696 (2008). Also featured in Nature Nanotechnology News and Views, BBC, New Scientist, Physics Today, Chemistry World, UK T&I.

9:20am **AS2+BI-ThM5 Single Biomolecule Force Spectroscopy Measurements; the Importance of Controlled Surface Chemistry**, *S. Allen,* The University of Nottingham, UK, ***S. Zauscher***, Duke University

Over the past decade, considerable interest has focussed on the ability of atomic force microscopy (AFM) and related techniques to record forces on or between single biological molecules. Many elegant examples are evident within the literature where such approaches have been employed, for example, for studies of force induced protein and RNA unfolding processes, and the dissociation of a wide-range of biomolecular complexes, including those involved in cell adhesion. Despite these elegant examples, and the considerable advantages of performing measurements at the single molecule level, such measurements are still far from routine. Indeed, even in the most experienced hands the experiments can suffer various problems which can arise from poor control of the chemistries employed for biomolecular surface attachment e.g. such as non-specific binding, difficulties in single molecule pickup and variability between experiments.

This presentation will provide an overview of the approaches we have employed in recent studies to address such issues. This for example, will include our studies of the mechanical properties of long molecules of DNA (>1000 base pairs) in which we have demonstrated the advantages of the addition specific terminal functionalities for surface attachment [1]. The advantages will be highlighted through our investigations of the effects on DNA mechanical properties of a range of different DNA binding molecules (e.g. drugs and proteins involved in DNA replication [1,2]). For studies of the dissociation of biomolecular complexes, we will highlight the advantages of an alternative approach, in which we have exploited the unique properties of dendron functionalized surfaces. Developed in collaboration with the group of Professor J.W. Park (Pohang University of Science and Technology (POSTECH), we have recently employed such surfaces for studies of DNA hybridization [3], and interactions between intracellular signal transduction proteins [4].

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[4] I.H. Kim, H.Y. Lee, H.D. Lee, Y.J. Jung, S.J.B. Tendler, P.M. Williams, S. Allen, S.H. Ryu, and J.W. Park (2009) *Analytical Chemistry* 81(9), 3276-3284

9:40am **AS2+BI-ThM6 Nanoarrayed Biomolecular Recognition followed by AFM**, ***P. Lisboa***, *L. Sirghi, A. Valsesia, P. Colpo, F. Rossi*, JRC-European Commission, Italy

The use of nanoarrayed surfaces in the field of biomolecular recognition is very promising for the improvement of bio-detection performance. Atomic Force microscope (AFM) is widely used to produce and characterize nanoarrayed surfaces and to carry out studies in the biological field. This work presents the study and characterization by AFM measurements of the fabrication steps of nanoarrayed surfaces based on organothiols (carboxylic and Polyethylene oxide) and the study of an immunoassay performed on these surfaces. The immunoassay was based on the bio-recognition between Human IgG/anti-Human IgG.

The nanoarray was fabricated by plasma colloidal lithography following the procedure developed in our lab. AFM studies of the process of nanoarray fabrication showed that during lithographic process, the etching step is crucial for the final the characteristics of the surface and that the process originates a good chemical nano-contrast.

The AFM analysis of the bio-interaction was performed after Human IgG immobilisation and anti-Human IgG recognition steps. The nanoarray was incubated with Human IgG solution resulting in an increase of height on the nano areas. The AFM image demonstrates that IgG molecules are adsorbed mainly on the border between the two organothiols. The preferential disposition of proteins on the borders of the two different materials with hydrophobic and hydrophilic groups was already reported and associated to the fact that the proteins tend to adsorb where they can find better accommodation. In our case, this effect can be explained by the fact that having the carboxylic spots with hydrophilic character the IgG hydrophobic groups are better accommodated on the border between the two materials, leading to higher adsorption on the boundaries. After the Human IgG incubation, the surface was blocked with BSA and the following step consisted in the immobilisation of anti-Human IgG. After this step, a height increase on the border of the COOH nano area is detected by AFM. The increase is about the double of the initial with Human IgG. This is an indication that the anti Human IgG binds specifically with the Human IgG already on the surface. The distribution on the borders of the nano-area can explain the better efficiency of the nano-patterns in biomolecular recognition as already described on different studies. The preferential proteins immobilisation on the nano areas boundaries seems to improve the binding efficiency of the immobilised Human IgG bio-detector by a better availability of the binding sites on the surface and reduction of steric hindrance.

10:40am **AS2+BI-ThM9 Deciphering Nanoscale Interactions: Artificial Neural Networks and Scanning Probe Microscopy**, *S. Jesse, M.P. Nikiforov, O. Ovchinnikov*, ***S.V. Kalinin***, Oak Ridge National Laboratory

Scanning Probe Microscopy techniques provide a wealth of information on nanoscale interactions. The rapid emergence of spectroscopic imaging techniques in which the response to local force, bias, or temperature is measured at each spatial location necessitates the development of data interpretation and visualization techniques for 3- or higher dimensional data sets.

In this presentation, we summarize recent advances in applications of neural network based artificial intelligence methods in scanning probe microscopy. The examples will include biological identification based on the dynamic of the electromechanical response, direct mapping of dynamic disorder in ferroelectric relaxors, and reconstruction of random bond-random field Ising model parameters in ferroelectric capacitors. The future prospects for smart multispectral SPMs are discussed.

Research was supported by the U.S. Department of Energy Office of Basic Energy Sciences Division of Scientific User Facilities and was performed at Oak Ridge National Laboratory which is operated by UT-Battelle, LLC.

11:00am **AS2+BI-ThM10 Scanning Probe and Differential Interference Contrast Imaging Methods for Studying Adhesion-Induced Tension and Membrane Fluctuation of Red Blood Cells**, ***N. Farkas***, *H. Kang,* National Institute of Standards and Technology*, F. Tokumasu,* NIAID, NIH*, J. Hwang, J.A. Dagata*, National Institute of Standards and Technology

Red blood cell (RBC) membrane fluctuation mediated by cooperative relationship between its cytoskeleton and lipid bilayer plays an important role in protein dynamics that is indicative of structural-functional properties of healthy or diseased RBCs. Probing of this characteristic membrane behavior requires dynamic interrogation of RBCs under physiological conditions by high-resolution, noninvasive microscopy techniques for which RBCs are required to be immobilized on a substrate while maintaining their viability. Therefore, detailed understanding of the adhesion process and its consequence on RBC shape and dynamic membrane response is critical. In the present study, we demonstrate our ability to engineer substrates with tunable surface zeta potential (SZP) for precise control of RBC adhesion. Specifically, 10 nm gold nanoparticles are adsorbed on poly-L-lysine coated cover slips as a compliant layer to locally modify the non-specific interaction between RBC membrane and substrate. By combining scanning probe microscope (SPM) and differential interference contrast (DIC) imaging techniques we develop a quantitative measurement methodology to investigate the relationship between attachment strength, RBC morphology, cell vibration and membrane fluctuation on these charge and topographically modulated substrates. Adhesion-induced tensing of the RBC membrane on modified substrates leads to changes in cell shape and functionality as determined by SPM force-volume and DIC monitoring of membrane dynamics. The substrate preparation and measurement methods presented here provide a feasible platform to obtain structure-function relationships of viable RBCs under physiological conditions and with that allow us to investigate dynamic behavior of RBCs and their response to diseases.

11:20am **AS2+BI-ThM11 Effect of Different Cations on the Nanomechanical Response of a Model Phospholipid Membrane : A Force Spectroscopy Study**, ***L. Redondo-Morata***, *G. Oncins, F. Sanz*, University of Barcelona, Spain

Understanding the effect of mechanical stress on biological membranes is of fundamental importance in biology since cells are known to naturally perform their function under the effect of a complex combination of forces. The chemical composition of such membranes is the ultimate responsible for determining the cellular scaffold, closely related to its function. Nevertheless, there is another factor that has been widely discussed in theoretical works but never experimentally tested in an accurate way, which is the presence of ions and their nature (radius and charge) on the stability of the bilayers. Micro-scale assays have revealed a wealth of information regarding the overall membrane mechanical resistance. Nonetheless, the diversity in the chemical composition of such membranes makes it difficult to individually probe the mechanical contribution of every particular membrane component. Here we use force spectroscopy to quantitatively characterize the nanomechanical resistance of supported lipid bilayers as a function of ionic strength and the composition of the electrolyte thanks to a reliable molecular fingerprint that reveals itself as a repetitive jump in the approaching force curve, hallmark of bilayer rupture. By systematically testing a set of bilayers composed of different phospholipids immersed in electrolytes composed of a variety of monovalent and divalent cations, we first show that the cationic radius, its charge density and the hydration number have an independent and important contribution to the overall bilayer mechanical stability. This work opens up avenues for characterizing the membrane (nano)mechanical stability and to assess the effect of different ions in the structure of the bilayers in an experimental and reproducible way.

Inkjet Technology: Printing, Materials Processing, and Microfluidics Fundamentals Topical Conference
Room: B3 - Session IJ+BI+MN+SE+AS-ThM

Inkjet Technology: Novel and Emerging Applications
Moderator: C. Klapperich, Boston University

8:00am **IJ+BI+MN+SE+AS-ThM1 An Overview of the Use of Ink-jet Technology for Non-traditional and Emerging Applications**, ***D.B. Wallace***, MicroFab Technologies, Inc. **INVITED**

In the last decade ink-jet printing technology has come to be viewed as a precision microdispensing tool. Today, this tool is being used in a wide range of manufacturing and instrument applications. Manufacturing applications include electrical (solders & nanometal conductors) & optical (microlenses & waveguides) interconnects; sensors (polymers & biologicals); medical diagnostic tests (DNA, proteins, cells); drug delivery (microspheres, patches, stents); scaffolds for tissue engineering; nanostructure materials deposition; and MEMS (Micro-Electrical-Mechanical) devices and packaging. Instrument applications using ink-jet technology have received less notice than manufacturing applications, but represent a growing class. Applications include protein identification (peptide mass fingerprinting, ion mass spectrometry tissue imaging) and structure analysis (protein crystallization); laser surgery and machining;

medical diagnostic instruments; extreme ultra-violet (EUV) radiation generation; and explosive detector calibration. This paper illustrates some of the manufacturing and instrument applications of ink-jet technology.

8:40am **IJ+BI+MN+SE+AS-ThM3 Inkjet Printing for Bioengineering Applications**, ***T. Boland***, Clemson University **INVITED**

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

9:20am **IJ+BI+MN+SE+AS-ThM5 Inkjet Printing for MEMS Fabrication**, ***J.A. Kubby***, *O. Azucena,* University of California, Santa Cruz, *C.L. Goldsmith, D. Scarbrough,* MEMTronics Corporation, *A.S. Mangalam*, Tao of Systems Integration, Inc. **INVITED**

In this presentation we will review the use of inkjet printing to fabricate Micro-Electro-Mechanical Systems (MEMS). We are investigating the use of sintered silver nanoparticle inks for the structural layer and polymers for the sacrificial layer in printed MEMS fabrication. As an example, inkjet printing technology has been used to fabricate microwave transmission lines for an RF MEMS switch on a glass substrate (with MEMTronics Corporation). 50 mm resolution was obtained using 10 pL drop volumes on a Corning 7740 glass substrate. The conductivity of the sintered silver structures were 1/6 that of bulk silver after sintering at a temperature much lower than the melting point of bulk silver. A comparison of the DC resistance of the sintered silver shows that it can match the performance for electroplated and etched copper. Printed coplanar lines demonstrated losses of 1.62 dB/cm at 10 GHZ and 2.65 dB/cm at 20 GHz. We will also discuss printing MEMS hot-wire anemometer sensors for use in aeronautical applications (with Tao of Systems Integration).

10:40am **IJ+BI+MN+SE+AS-ThM9 Formation and Surface Characterisation of a Combinatorial Acrylate Polymer Microarray Produced by an Ink-Jet Printer**, ***A.L. Hook***, *J. Yang,* University of Nottingham, UK, *D.G. Anderson, R.S. Langer,* Massachusetts Institute of Technology, *M.C. Davies, M.R. Alexander*, University of Nottingham, UK

Polymer microarrays are emerging as a key enabling technology for the discovery of new biomaterials. This platform can readily be screened for properties of interest and for correlating surface chemistry with biological phenomenon. A method for forming polymer microarrays has been developed whereupon a contact printer is used to deposit nanolitre volumes of premixed acrylate monomer and initiator to defined locations of a glass slide with subsequent UV irradiation[1]. This results in polymerisation occurring on the slide, offering a useful high throughput materials discovery platform. The identification of relationships between cell response to these materials and surface properties is facilitated by high throughput analysis of this slide format[2,3]. Here, we have formed these polymer microarrays for the first time using ink-jet printing, to offer flexibility of slide production. Characterisation was achieved using a high throughput surface analysis approach, including the techniques of X-ray photoelectron spectroscopy, time-of-flight secondary ion mass spectroscopy and sessile drop water contact angle measurements[2]. Of particular interest were polymers containing ethylene glycol functionality that were investigated for their switchable properties under biologically relevant conditions.

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11:00am **IJ+BI+MN+SE+AS-ThM10 Development of an Inkjet Printed Drug Formulation**, ***N. Scoutaris***, *C.J. Roberts, M.R. Alexander,* Nottingham University, UK, *P.R. Gellert*, AstraZeneca, UK

The potential application of ink-jet printing technology as a novel drug formulation technique is examined in this study. Since the inkjet printing technology offers high accuracy of fluids, a success implementation of the project can offer the capability to produce precise amounts of medicines, tailored for each patient.

Felodipine, an antihypertensive drug, was used as an example of an active pharmaceutical ingredient (API), and polyvinyl pirrolidone (PVP) as an excipient. These were dissolved at various ratios in a mixture of ethanol and DMSO (95/5). Using a piezoelectric driven dispenser, picolitre size droplets of the solutions were dispensed onto suitable hydrophobic substrates. The dried products were characterized using AFM, localized nano-thermal analysis and high resolution vibrational spectroscopy (ATR-IR and Raman). Results indicate intimate mixing of the micro-dot API and excipient mixtures. Specifically, ATR-IR confirmed the interaction of felodipine and PVP by means of hydrogen bonding. Nanothermal analysis indicates a single glass transition point which is lowered as the API concentration increases. Finally, confocal Raman microscopy mapping on single droplets allows the visualization of the homogeneous distribution of the drug. These results are a promising first step to ink jet printing of pharmaceuticals.

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11:20am **IJ+BI+MN+SE+AS-ThM11 Fabrication of Plastic Biochips via *in situ* Inkjet Oligonucleotide Array Synthesis**, ***I. Saaem***, *K. Ma, J. Tian*, Duke University

With the foreseeable integration of microfluidics and microarrays, polymers stand to play a critical role. Generally, arrays are constructed on glass, silicon, membranes, or polyacrylamide matrices. The preference of such materials makes the marriage of arrays and microfluidics fraught with challenges such as developing low-cost manufacturing methods and simultaneously scaling rapidly for diverse applications and chip designs. In addition, deposition or synthesis of the requisite biomolecule reliably in defined surface geometries is a challenging task. We try to alleviate these problems by utilizing the steadily maturing art of inkjet printing on polymer substrates. Polymeric, or plastic, biochips have several advantages in cost, durability, the ability to scale to industrial techniques and possibly serve as disposable point-of-care devices. In our studies, we utilized an inkjet based *in situ* oligonucleotide synthesis platform that uses salvaged printheads from commercial printers. The platform utilizes standard four-step phosphoramidite chemistry with some modifications in order to synthesize oligonucleotides on functionalized substrates. A sensitive pressurization system is used to ensure print quality and an on-board vision system enables substrate registration and synthesis monitoring. Using this platform we synthesized oligonucleotides on prepatterned functionalized plastic slides. Such patterned substrates help in proper droplet formation and fluid mixing on the surface while mitigating satellite and irregular drops, which can lead to cumulative synthesis errors. Functional integrity of synthesized oligonucleotides was confirmed by hybridization with complementary strands. Being able to hot emboss microfluidic structures directly onto plastic slides in combination with the ability to generate arbitrary sequences provides diagnostic capabilities as well as the means to harvest pools of cheap oligonucleotides on demand. Importantly, our results show that the combination of technologies presented is a suitable strategy of fabricating plastic biochips at a cost-effective industrial scale.

11:40am **IJ+BI+MN+SE+AS-ThM12 Study on the Effects of Particle Size and Substrate Surface Properties on the Deposition Dynamics of Inkjet-Printed Colloidal Drops for Printable Photovoltaics Fabrication**, *S. Biswas*, ***Y. Sun***, Binghamton University

Using fluorescence microscopy, the inkjet deposition dynamics of monodispersed polystyrene particles in the size range of 0.02 to 1.1 µm have been studied on glass, Ar plasma cleaned glass, and PDMS coated glass substrates. The results show that the substrate properties play an important role in determining the final dried patterns formed by the colloidal particles. Our observations also reveal that particle size and contact angle formed by the solvent in the dispersion determine how close to the contact line the particles can be deposited. It is found that the diameter of the dried deposited features decrease with the increase in hydrophobicity of the substrates, irrespective of particle sizes. On Ar plasma treated glass ($\theta_A = 13°$), the smaller particles (0.02 & 0.2 µm) show larger depositions than the bigger 1.1 µm particles. Similar type of behavior of the dried deposited features are also observed on clean glass samples ($\theta_A = 36°$). In contrast, on PDMS coated glass ($\theta_A = 111°$), the behavior of the contact line diameter with the evaporation of the drop is similar for all types of particles. On an average, the diameters of the dried deposited features on PDMS coated glass substrates are independent of particle sizes. This study can serve as a realistic experimental model system for a number of fundamental queries on how the final deposition microstructure depends on the ink formulation and substrate properties. The knowledge obtained here can be explored further to optimize process parameters for the fabrication of hybrid solar cells with improved morphology and device properties.

Thin Film
Room: B4 - Session TF-ThM

Nanostructuring Thin Films II
Moderator: P.D. Rack, University of Tennessee

8:00am **TF-ThM1 Nanostructure of Gold Film Over Vertically Aligned Carbon Nanofiber Surface Impact on Immobilization of Alkanethiols**, ***R. Desikan***, North Carolina State University, *T.E. McKnight*, Oak Ridge National Laboratory, *P.D. Rack*, University of Tennessee, *A.V. Melechko*, North Carolina State University

Chemical self-assembly has been widely used for several applications including surface modification and functionalization. The physics and chemistry of alkanethiol self-assembled monolayers (SAM) has been extensively studied. It is well known that SAM structures of alkanethiol are strongly influenced both by the surface structure of the underlying gold substrate. It has also been shown that the adsorption of alkanethiols onto gold surfaces having large, flat grains produces high-quality self-assembled monolayer. In this study we have investigated the effect of SAM formation over carbon nanofibers coated with gold thin film. The film nanostructure is strongly affected by the morphology of the surface on these high aspect ratio 3D features. The thiolated molecules are chosen due to the strong affinity of sulfur head groups with the gold surface of the carbon nanofibers. The difference in surface stress response alkanethiol of adsorption in vapor phase on large- and small-grained gold correlates well with differences in alkanethiol coverage and SAM structure on those substrates. We will present observations of how the surface structure of the underlying gold substrate influences the kinetics of SAM formation. It has been shown earlier that in the case of gold surfaces with small grain sizes (<100 nm), access to the high coverage, standing-up phase is inhibited. A comparison of annealed for increasing grained gold surfaces on the fibers to as deposited film will be discussed. The characterization of molecular coverage as a function of the gold grain size with an x-ray photoelectron spectroscopy will be presented.

8:40am **TF-ThM3 Spontaneous Growth of In-whiskers from In-Y Thin Films Prepared by Combinatorial Magnetron Sputtering Technique**, ***T. Takahashi***, *A. Abdulkadhim, D. Music, J.M. Schneider*, RWTH Aachen University, Germany

In-Y binary thin films with a composition gradient were prepared using a combinatorial magnetron sputtering technique. In-whiskers grow spontaneously from the film surface at room temperature upon exposure to air. Whisker morphology and population vary with the In to Y ratio. An appreciable amount of In-whiskers is formed at film compositions close to In-25 at.%Y. The In-whisker thickness ranges from a few hundreds nanometers to a few micrometers.

In order to identify the whisker growth mechanism, temporal changes of the film surface upon air exposure were captured using scanning electron microscopy. X-ray micro diffraction was employed for studying the structural evolution during the In-whisker growth in air. The results show that the In-whiskers grow not from the tip but from the root. The whisker growth rate was as high as 150 nm/s. The growth of In-whiskers is found to be related to the incorporation of oxygen into the film during air exposure. Correspondingly, the In concentration within the film decreases as In-whiskers grow. The mechanism of the spontaneous In-whisker growth presented here can be understood based on the stress-induced extrusion of In-whiskers due to the selective room temperature oxidation of Y in sputtered In-Y thin films.

9:00am **TF-ThM4 Photothermally Induced Microchemical Functionalization of Organic Monolayers**, ***A. Schroeter***, *B. Klingebiel, N.O. Hartmann*, University of Duisburg-Essen, Germany

Photochemical routines are widely recognized as a versatile means to fabricate multifunctional patterned organic monolayers with laterally alternating chemical terminations. The lateral resolution, in turn, usually is limited by optical diffraction, that is, the fabricated structures are not much smaller than the wavelength even when highly focusing optics are used. A simple routine for sub-wavelength nanopatterning of organic monolayers relies on photothermal processes, which take advantage of nonlinear effects [1]. In photothermal processing a focused laser beam is used to locally heat the substrate and initiate thermal decomposition of the monolayer. Silane-based monolayers can be patterned from the micrometer-range down to the sub 100 nm range. In analogy to photochemical routines, it is tempting to explore photothermal procedures which allow to locally functionalize organic monolayers. Here we report on a simple photothermal procedure for local bromination of organic monolayers [2]. Surface-oxidized silicon samples are coated with alkylsiloxane monolayers. Local irradiation with a focused beam of an argon ion laser (λ = 514 nm) in gaseous bromine leads to bromination of the monolayer in a confined micrometer-sized reaction zone. While irradiation induces photolysis of bromine molecules in the gas phase, the local temperature rise of the substrate in the focal area allows for bromination of the alkyl chains. Thus locally brominated surface structures are fabricated. In conjunction with other chemical transformations this provides an efficient route to a broad variety of functional groups. This allows to build up functional surface architectures via directed self-assembly of nanoscopic building blocks such as noble metal nanoparticles and stimuli-responsive polymers. Mechanistic aspects and prospects of photothermal routines in micro- and nanofabrication of multifunctional organic monolayers are discussed.

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9:20am **TF-ThM5 Composite Nanoparticles Produced Using Plasma-Enhanced Chemical Vapor Deposition of SiO_2 and Amine-Containing Films**, ***J.C. Shearer***, *E.R. Fisher*, Colorado State University

TiO_2 and Fe_2O_3 nanoparticles are of significant importance in both chemical and biological applications. TiO_2 nanoparticles are used in paint, coatings, food, solar technology and many other areas. Fe_2O_3, as well as other magnetic nanoparticles, are used in the biomedical industry in drug delivery schemes as well as for magnetic resonance imaging contrast agents. Silica-coated TiO_2 and Fe_2O_3 nanoparticles have distinct properties and enhanced functionality over those of uncoated nanoparticles. Plasma-enhanced chemical vapor deposition (PECVD) was employed to conformally coat TiO_2 and Fe_2O_3 nanoparticles with SiO_x and amine-containing films, thereby creating composite nanomaterials. Hexamethyldisiloxane (HMDSO)/O_2 plasmas were used to create SiO_2 and $SiO_xC_yH_z$–coated nanoparticles and pulsed hexylamine (HexAm) plasmas were used to create amine-containing hydrocarbon materials, all of which were analyzed using Fourier-transform infrared spectroscopy (FTIR), x-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), and magnetic susceptibility. Films deposited on the nanomaterials showed little difference from those deposited on flat substrates. The performance of the TiO_2 nanoparticles was tested using UV-vis spectroscopy to determine dispersion characteristics of SiO_x-coated TiO_2 materials, which can give information about the agglomeration of the nanoparticles in solution. Notably, the coated materials stay dispersed longer in polar solvents, suggesting the coated nanoparticles may be better suited for applications involving colloidal suspensions. Magnetic susceptibility characterized the magnetic properties of the Fe_2O_3 nanoparticles before and after film deposition. Comparison of the mechanical and chemical properties of different composite nanomaterials will be discussed along with the influence of film composition on performance.

9:40am **TF-ThM6 Tailoring Local Conductivity by the Formation of Ag Nanoparticles in SiO_2 Xerogel Films**, ***M.F. Bertino***, *R.J. Caperton, A.A. Baski*, Virginia Commonwealth University

Thin SiO_2 xerogel films (~200 nm) were fabricated by dip coating and were doped with Ag^+ by adding $AgNO_3$ to the parent solution. Nanoparticles were then fabricated inside the pores of these films by either exposing them to ultraviolet (UV) illumination or by locally injecting charge using a conducting atomic force microscope (CAFM). In both cases, reduction of the Ag^+ ions to the metallic state and the subsequent formation of Ag nanoparticles was observed by optical absorption spectroscopy and X-ray diffraction. Surprisingly, the formation of these Ag nanoparticles was accompanied by a decrease in the electrical conductivity of the films. For exposed regions with nanoparticle formation, CAFM measurements demonstrated no measurable current (< 1 pA) at sample bias voltages above 10 V. We attribute this decreased conductivity to a change in the morphology of the conducting Ag species in the film. Before reduction, Ag^+ ions are attached to negatively charged pore walls in a comparatively dense packing and produce a conducting film. After reduction with UV exposure or CAFM charge injection, the silver metal agglomerates into conducting nanoparticles that no longer form a percolated network, leading to insulating behavior. Local modification of the conductivity on the nanometer-scale is possible by operating the CAFM with an applied voltage above 6 V and scanning a defined pattern. Subsequent imaging of the area at lower voltage then shows insulating behavior in the previously patterned regions. Larger-scale patterning on the micron-to-mm scale is possible by utilizing a mask when exposing the film to UV illumination. Extensions of this method to the fabrication of photonic and plasmonic devices is being explored.

10:40am **TF-ThM9 Synergistic Ag (111) and Cu (111) Texture Evolution in Phase Segregated $Cu_{1-x}Ag_x$ Magnetron Sputtered Composite Thin Films**, ***D.I. Filoti***, *A.R. Bedell, J.M.E. Harper*, University of New Hampshire

We investigated the texture and microstructure evolution of $Cu_{1-x}Ag_x$ composite thin films through x-ray diffraction pole figures as a function of composition for $x \leq 0.5$. As-codeposited at room temperature by magnetron sputtering, the fcc composite $Cu_{1-x}Ag_x$ emerge as a phase-segregated thin film, when the Ag volume fraction represents more than 15 at. % up to 50 at. %, or as a single phase thin film when Ag volume fraction represents less than 15 at. %. The texture evolution of Cu (111) and Ag (111) in phase-segregated Cu-Ag thin films proves to be synergistically enhanced when compared to pure copper or silver thin films. Not only is a stronger perpendicular (111) fiber texture obtained, but also an in-plane alignment of Ag (200) develops related to deposition direction and composition. By the use of transmission electron microscopy we observed a decrease in grain size in Cu-Ag composite films as compared with pure copper and silver films. These Cu-Ag thin films are being evaluated for antimicrobial applications.

11:00am **TF-ThM10 Plasmonic Phenomena in Indium Tin Oxide and ITO-Au Hybrid Films**, *S. Franzen, C. Rhodes, M. Cerruti, R.W. Gerber,* ***M. Losego***, *J.-P. Maria, D.E. Aspnes*, North Carolina State University

For more than 100 years the plasmonic periodic table has been dominated by two elements, Ag and Au. The change in the surface plasmon polariton (SPP) signal in Au thin films is currently one of the most widely used methods for detecting binding interactions in biological systems. Despite broad interest, there has been sparing fundamental research into new plasmonic materials. Here, we elucidate some equivalences regarding plasmonic phenomena in conducting metal oxides, specifically indium tin oxide (ITO), and Au. In contrast to Ag and Au, conducting metal oxides offer the possibility of tuning both the location of the metallic resonance and its width according to deposition conditions. We investigate properties of ITO and ITO/Au layers by reflectance spectra R_p and R_s obtained for light polarized parallel and perpendicular, respectively, to the plane of incidence. Data were obtained in the Kretschman configuration. These data reveal two distinct types of plasmonic phenomena, one due to a capacitive-type oscillation that can only occur for very thin conducting films and the second being the usual surface plasmon resonance. One or the other can be realized either by changing the ITO thickness or by depositing either metallic or nanostructured Au on the ITO to change boundary conditions. The results can be understood completely through a combination of the Drude model for free carriers in a metal, Bruggeman effective-medium theory, and the Fresnel equations. This provides a new dimension for engineering plasmonic phenomena for investigations of molecules adsorbed at interfaces.

11:20am **TF-ThM11 Micro and Nanostructured Interfaces for Therapeutic Delivery**, ***T.A. Desai***, University of California, San Francisco **INVITED**

Efficient drug delivery remains an important challenge in medicine. Continuous release of therapeutic agents over extended time periods and in accordance to a pre-determined temporal profile; local delivery at a constant rate to overcome systemic toxicity; improved ease of administration, and increasing patient compliance are some of the unmet needs of the present drug delivery technology. This talk will discuss in vivo drug delivery strategies that capitalize on the strengths of micro and nanofabrication. By taking advantage of our ability to control topography and chemistry at submicron size scales, we have developed organic and inorganic interfaces which modulate cell function while at the same time allow for controlled drug release kinetics. Due to our ability to create monodisperse features (pores, wires, and hollow tubes)as small as several nanometers in a variety of non-planar biocompatible materials, these interfaces offer advantages in their reproducibility, stability, and their ability to intimately contact cell and tissue surfaces. Such nanoengineered interfaces may be optimized for biomolecular selectivity and surface bioactivity, leading to unique interfacial properties not achieved through existing drug delivery approaches. Nanotechnology can add functionality to current drug delivery platforms while becoming an enabling technology leading to new basic discoveries in the biological sciences.

Biomaterial Interfaces
Room: K - Session BI+AS+NS-ThA

Micro and Nanoengineering of Biointerfaces I
Moderator: G.J. Leggett, University of Sheffield

2:00pm **BI+AS+NS-ThA1 Spatial Organization and the Mechanics of Signal Transduction in Cell Membranes**, ***J.T. Groves***, University of California, Berkeley **INVITED**

Signal transduction in living cells is carried out through cascades of chemical reactions, which generally begin on the cell membrane surface.

In recent years, there has been growing realization that the large-scale spatial arrangement of cell surface receptors can regulate the outcome of ensuing signal transduction process. Signaling through the T cell receptor (TCR) in the context of the immunological synapse provides a case in point. Spatial reorganization of TCRs occurs on multiple length-scales, and apparently with multiple purposes, during antigen recognition by T cells. The cell membrane and cytoskeleton, working as an inseparable unit in this case, create the mechanical framework within which TCR signaling processes occur. To better study these phenomena, a new experimental strategy, in which the spatial positions of cell membrane receptors are directly manipulated through mechanical means, has emerged. By physically inducing a 'spatial mutation' of the signali ng apparatus, the role of spatial organization in signal transduction as well as the mechanisms by which it arises can be illuminated. Specific applications of this strategy to TCR signaling and other cell-cell signaling systems will be discussed.

2:40pm **BI+AS+NS-ThA3 Investigation of Array Spotting of Polymer Supported Lipid Bilayers**, ***S. Kaufmann***, *M. Homenuke,* ETH Zurich, Switzerland, *J. Sobek,* University of Zurich, Switzerland, *E.O. Reimhult, M. Textor*, ETH Zurich, Switzerland

Supported lipid bilayers (SLB) constitute a simple model of cell membranes and are of particular interest as components of future generations of biosensors based on transmembrane proteins. Techniques which are able to produce arrays with small micrometer-sized sensor areas in a cheap and fast way are beneficial. A major challenge producing such arrays of SLBs is their need for an aqueous environment during formation and operation, which has so far prevented the wide-spread use of common techniques to produce arrays such as spotting.

Poly(ethylene glycol) (PEG) can be incorporated into the membrane of liposomes through lipid molecules end-functionalized with a PEG chain and these liposomes were shown to spontaneously fuse to PEG-SLB on glass surfaces with a highly hydrated PEG cushion on each side of the membrane allowing ample space and protection for incorporation of membrane proteins[1,2]. Since Cremer and coworkers [3] also showed that with increasing PEG concentration the air-stability of PEG-SLBs increases it is a very promising SLB system to use for spotting where membrane air exposure during processing is a severe constraint.

We present an investigation of the limits to spontaneous PEG-SLB in terms of PEG-lipid density, demonstrating that crossing the mushroom-to-brush regime of polymer concentration prevents the PEG-SLB formation due to steric effects and shielding of the interactions[2]. Furthermore, we present conditions under which formation of PEG-SLBs is facilitated and can proceed by liposome fusion also in the brush regime as well as characterization of the kinetics of formation and the structure of these PEG-SLBs. The use of such buffers and liposomes for production of membrane arrays on glass using a non-contact piezo-spotter was then explored in detail in order to find optimal conditions of buffer composition and PEG concentration.

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3:00pm **BI+AS+NS-ThA4 Direct Laser Patterning of Soft Matter: Photothermal Processing of Supported Phospholipid Multilayers with Nanoscale Precision**, ***M. Mathieu***, *D. Schunk, S. Franzka, C. Mayer, E. Hasselbrink, N.O. Hartmann*, University of Duisburg-Essen, Germany

Supported phospholipid bilayers and multilayers are widely recognized as model systems of biological membranes. Recently, these coatings have also gained significant attention as flexible biomolecular matrixes in various micro- and nanofabrication schemes [1]. Here, we report on direct laser patterning of supported phospholipid bilayer stacks. Direct laser patterning techniques are widely recognized as powerful tools in rapid prototyping and small volume fabrication. They offer a high flexibility in fabrication of complex 2D structures and patterning can be carried out at fast writing speeds over macroscopic length scales at ambient pressures or even in liquids [2]. For patterning multi-layered dioleyl-phosphatidic acid (DOPA) films were deposited on native silicon samples via spin coating. Then photothermal processing with a focused laser beam at λ = 514 nm is used for removal of the coating at predefined positions without causing any significant change in adjacent areas. Moreover, processing with nanoscale precision is feasible despite the soft and fluid nature of phospholipid films. In particular, holes with diameters from 1.8 µm down to 300 nm and below are fabricated using a laser spot diameter of about 2.5 µm [3]. Furthermore, partial removal can be carried out at incremental steps leaving a distinct number of bilayers behind. The underlying nonlinear dependence of the patterning process on the laser intensity is traced back to the interplay between the laser-induced transient local temperature rise and the thermally activated desorption of the phospholipid molecules. Generally, the lateral resolution in photothermal processing depends on the thermal and chemical stability of the coating. Phospholipid films, of course, are soft supramolecular assemblies. Despite their soft nature, however, the collective interactions are quite strong. This gives rise to a strong nonlinearity as observed here. Considering these features, photothermal laser processing constitutes a powerful tool for micro- and nanopatterning of phospholipid films.

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2. D. Dahlhaus, S. Franzka, E. Hasselbrink, N. Hartmann, Nano Lett. 6 (2006) 2358.

3. M. Mathieu, D. Schunk, S. Franzka, C. Mayer, E. Hasselbrink, N. Hartmann, Small, accepted.

3:40pm **BI+AS+NS-ThA6 The Role of Liposomes in Fluorescent Based Microarrays: From Surface Immobilization of Membrane Proteins to Highly Fluorescent Labels**, ***M. Bally,*** ETH and University Zurich, Switzerland, *K. Bailey,* CSIRO, Australia, *S. Syed, S. Buergel, J. Voeroes*, ETH and University Zurich, Switzerland

Technologies utilizing arrays of immobilized biomolecules on planar surfaces are emerging as powerful high throughput tools for bioanalytical measurements. Nowadays, optical sensors based on fluorescence detection are the most widespread. However, many applications especially in the area of protein sensing, rely on the availability of optimized sensing interfaces and signal amplification strategies. Liposomes, due to their hollow particle-like structure and their unique chemical and physical properties, have greatly contributed to the development of sensitive and accurate biological assays.

In this presentation, we highlight with results obtained recently, the contributions of phospholipid vesicles to the development of high performance fluorescence based biosensors.

First, liposomes are an optimal platform for the surface immobilization of membrane proteins since they provide the natural environment required for the functional surface immobilization of these fragile molecules. We demonstrate the creation of a functional, heterogeneous array of G-protein coupled receptors. Vesicles obtained from cellular membrane extracts containing either the H1R-histamine receptor or the M2R-muscarinic receptors were immobilized on a conventional oligonucleotide microarray via complementary tags. Fluorescent ligand binding assays were then performed illustrating that the receptors kept their native conformation. As an alternative platform, we introduce a novel approach for the creation of vesicle multilayers using zirconium phosphate chemistry. As demonstrated in a model biomolecular binding assay, such three dimensional constructs increase the protein loading capacity of a sensor surface.

Liposomes are also excellent candidates as labels for biological assays: phosphocholine-based vesicles are non-fouling and biomolecules or marker molecules (e.g. fluorophores or enzymes) can be easily attached to their surface or encapsulated in their inner cavity. We show that fluorescently labeled phospholipid vesicles provide simple and cheap means for signal amplification and sensitive protein detection on a microarray format. Using vesicles, up to 100 fold increase in sensitivity was observed in a model protein microarray with confocal read-out, compared to a conventional assay performed with fluorophore labeled antibodies.

The various approaches presented here will contribute to the development of sensitive and high performance microarrays for a variety of applications including the investigation of membrane proteins.

4:00pm **BI+AS+NS-ThA7 Fusion of Biomimetic 'Stealth' Probes into Lipid Bilayer Cores**, ***B. Almquist***, *N. Melosh*, Stanford University

The ability to specifically and non-destructively incorporate inorganic structures into or through biological membranes is essential to realizing full bio-inorganic integration, such as arrayed on-chip patch-clamps, drug delivery, and biosensors. However, molecular delivery and interfaces to inorganic objects, such as patch-clamp pipettes, generally rely upon destructive formation of membrane holes and serendipitous adhesion, rather than selective penetration and attachment to the bilayer. In fact, materials greater than a few nanometers in size have not been shown to penetrate lipid bilayers without disrupting the continuity of the membrane. In this talk, I will discuss the development of nanofabricated probes that spontaneously insert into the hydrophobic membrane core by mimicking the hydrophobic banding of transmembrane proteins, forming a well-defined bio-inorganic lateral junction. These biomimetic 'stealth' probes consist of hydrophilic posts with 2-10 nm hydrophobic bands formed by molecular self-assembly, and are easily fabricated onto a variety of substrates including silicon wafers, nanoparticles, and atomic force microscope (AFM) tips.

By fabricating this architecture onto AFM probes, we have directly measured the penetration behavior and adhesion force of different molecular functionalities within the bilayer. It has been found that following insertion, the stealth probes remain anchored in the center of the bilayer, while purely hydrophilic probes have no preferred location. The strength of the stealth probe adhesion varies greatly between short and long chain alkane functionalizations, indicating that chain mobility, orientation, and hydrophobicity all contribute to molecular stability within the bilayer. In addition, the consequences of geometric factors such as band thickness and the presence of multiple bands on interface stability have been established. By selectively choosing the desired properties of the hydrophobic band, it will be shown that it is possible to tune the failure tension of the interface from values comparable to that of pristine lipid vesicles to only a fraction of the strength. Finally, the ability to transfer the stealth probe behavior to other platforms (e.g. nanoparticles for drug delivery) will be discussed.

4:20pm **BI+AS+NS-ThA8 Seeing Nanopore-spanning Supported Lipid Bilayers**, ***K. Kumar***, *S. Kaufmann, M. Textor, E.O. Reimhult*, ETH Zurich, Switzerland

Supported lipid bilayers (SLBs) present a highly interesting cell-membrane-like format to study sensitive ion channels or other membrane proteins. If formed by the rupture of liposomes, they then have the major advantage over other planar membrane architectures for biosensing in that they can be formed completely bereft of organic solvents by self-assembly, enabling the further incorporation of the aforementioned sensitive membrane proteins.[1] Porous structures allow the use of fusogenic surfaces which enhance the formation of SLBs from liposomes, while accommodating the incorporation of larger membrane proteins by decoupling them from the surface. We have developed a particle lithography process to produce high aspect ratio pores with a diameter tunable between 40 nm and 200 nm into silicon nitride, silicon oxide or titanium oxide to take advantage of a range of different surface chemistries. SLBs were formed on these porous substrates and characterized by fluorescence and force microscopy. These results suggest that the underlying nanotopography of the substrate plays a major role in both the formation and characterisation of nanopore-SLBs. The outcome of liposome adsorption is strongly influenced by roughness features in the same size range as the liposomes, which has important implications for the reproducible formation and characterization of nanopore-spanning planar lipid membranes necessary for future applications in integrated membrane-based sensing.

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4:40pm **BI+AS+NS-ThA9 Formation of Protein Surface Patterns by Ligand Self-Selection *from Mixed Protein Solutions***, *M. Dubey,* University of Washington, *K. Emoto,* Great Basin Scientific, *H. Takahashi, D.W. Grainger,* University of Utah, ***D.G. Castner***, University of Washington

Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) has been shown to be very sensitive for surface analysis and has been very useful for trace element detection. With the advent of improved analyzers, imaging ToF-SIMS provides spatial distribution of different species and helps in plotting the surface reactivity maps. Use of multivariate analysis, especially Principal Component Analysis (PCA) makes this technique even more powerful by differentiating regions with different chemistries. ToF-SIMS and PCA has been used in this work to study a very important two component patterned chemistry, which can have applications in bio-chips and cell-based biosensors. The chemistry is based on n-hydroxysuccinimide (NHS) esters; these molecules are widely used as leaving groups to activate covalent coupling of amine-containing biomolecules onto surfaces. The present work utilizes our knowledge of a previously studied model system, where NHS molecules were self-assembled on a gold substrate, and XPS and ToF-SIMS was used to characterize and understand the effect of hydrolysis and regeneration. We have demonstrated the extension of this chemistry to a commercial poly(ethylene glycol) (PEG)-based polymer films coated on glass slides. NHS and methoxy-capped regions were co-patterned onto these slides using photolithographic methods; then imaged with ToF-SIMS/PCA. NHS surface reactive zones are clearly resolved at high sensitivity despite the complexity of the matrix chemistry. Surface-specific protein coupling was observed by surface-selective reaction of streptavidin with the NHS patterns. The next step involved the preparation of photolithographic patterns of two affinity ligands (biotin and chloroalkane) for the specific immobilization of two different proteins (Streptavidin and HaloTag®). Spontaneous formation of high-fidelity surface patterns of the two proteins from their mixed solution was observed and characterized. In addition to Streptavidin and HaloTag®, ToF-SIMS detected the presence of non-specific BSA adsorption, a masking protein present in excess in the protein solutions, onto the patterned surfaces. ToF-SIMS amino acid-derived ion fragment yields summed to produce surface images can reliably determine which patterned surface regions contain bound proteins, but do not readily discriminate between different co-planar protein regions. However PCA of the ToF-SIMS data, improves discrimination of ions specific to each protein, facilitating surface pattern discrimination based on protein type. Also, ToF-SIMS imaging detected regions where residue from incompletely removed UV-exposed photoresist was present and its influence on protein adsorption.

5:00pm **BI+AS+NS-ThA10 Fabrication of Protein Patterns by Direct Electron-Beam Writing in a Protein-Repelling Template**, *N. Ballav,* Universität Heidelberg, Germany, *H. Thomas, T. Winkler, A. Terfort,* Goethe-Universität Frankfurt, Germany, ***M. Zharnikov***, Universität Heidelberg, Germany

One of the challenges of modern nanotechnology is the development of reliable, efficient, and flexible methods for the fabrication of ordered and complex patterns comprised of different proteins. An essential element of almost all available approaches is a protein-repelling "background" matrix, surrounding the active protein-adsorbing areas – the matrix prevents adsorption of proteins beyond these areas. Such a matrix is usually comprised of oligo- or poly(ethylene glycol)-based materials and is generally prepared by a backfilling procedure after the fabrication of the protein-attracting patterns. We present an alternative approach, showing that the protein-repelling matrix, both SAM- and polymer-like, can be used as a primary template for *direct* electron-beam writing of both *non-specific* and *specific* protein patterns of *any desirable shape*, including gradient ones, on a flexible length scale. The above factors make the approach quite versatile, which is additionally strengthened by intrinsic flexibility of electron-beam lithography, a wide range of suitable electron energies, broad availability of commercial oligoethylene glycol compounds, variable substrate material, and flexible choice of the target proteins. Complex gradient patterns fabricated by the suggested approach can become an important tool for mimicking natural biological interfaces which frequently possess gradient character – a typical way of encoding and displaying directional biological information.

BioMEMS Focus Topic
Room: A8 - Session BM+MN+MS+TF+BI-ThA

Advances in Microfluidics for BioMEMS
Moderator: G.W. Rubloff, University of Maryland

2:00pm **BM+MN+MS+TF+BI-ThA1 Advances towards Programmable Matter**, ***D. Erickson***, Cornell University **INVITED**

A dichotomy exists between the bottom-up self-assembly paradigm used to create regular structures at the nanoscale, and top-down approaches used to fabricate arbitrary structures serially at larger scales. The former of these enables rapid, highly parallel assembly but lacks critically important features of the latter such as the ability to arbitrarily direct the assembly location and perform error correction. We and our collaborators have recently proposed an alternative approach which combines these two based on dynamically programmable self-assembling materials, or ***programmable matter***. The uniqueness of our approach is that it uses dynamically-switchable affinities between assembling components facilitating the assembly of irregular structures. In this talk I present an overview of our approach and detail some of the analytical and experimental advances towards a programmable matter system we have recently made. These include: the development of a multi-chamber microfluidic chip for improved far-field assembly, the demonstration of near-field inter-tile

affinity switching using a thermorheological assembly fluid and the ability to enhance assembly in three dimensions using unique fluid-structure interactions.

2:40pm **BM+MN+MS+TF+BI-ThA3 A Multilayered Microfluidic System with Buried Channels and Cell Compartmentalization for Engineering Heterogeneous Neural Networks***, C. James, A. Greene,* ***A. Schiess****, G. Bachand,* Sandia National Laboratories*, M. Romero-Ortega*, University of Texas at Arlington

Current technology for engineering *in vitro* neural networks utilizes cell guidance cues that yield only temporary networks (< 1 month) as the cells rapidly diverge from their designed guidance cues during development of the culture. In addition, these engineered networks are typically comprised of homogeneous populations of neurons, thus the lack of multiple neuron types produces oversimplified networks that do not adequately represent in vivo networks. In addition, effective control over synaptic connections between different populations of neurons has not been demonstrated. Here, we describe a novel hybrid technology of multi-layered microfluidics with compartmentalized chambers containing multiple neuron types for engineering robust and complex neural networks with high resolution organization of synaptic connections. The device contains a first level of microfluidic channels etched 1-2 microns into the base glass substrate. These channels are fabricated with a novel process using a silicon nitride mask for hydrofluoric acid undercut etching to create buried microfluidic channels for robust containment and guidance of neurons. After the etching process, photoresist liftoff is performed to selectively adsorb poly-L-lysine (PLL) within the buried channels for improved neuron attachment and outgrowth at pre-defined locations. Polarity control of neurons is provided through a continuous set of guidance cues to promote axon development, while interrupted sets of guidance cues promote dendrite development. Current results show that axons and dendrites are positioned at predefined locations with a >65% accuracy. A second level of microfluidic channels and large (~mm) cell chambers are fabricated in polydimethylsiloxane (PDMS) from two-level SU-8 master molds. The base glass substrate and the PDMS substrate are aligned and bonded to create interconnects between channels in both substrates. These interconnects provide interaction regions for the development of synapses between growing neurites from cells in different chambers. We are currently applying this technology to engineer corticostriatal networks, an important region of the brain responsible for integrating multiple informational inputs crucial to complex decision-making in higher mammals. Specifically, we are using patch-clamp electrophysiology to track the development of synaptic memory in the form of long-term depression and potentiation (LTD/LTP) in these engineered networks.

3:00pm **BM+MN+MS+TF+BI-ThA4 Vesicle Production on a Microfluidic Platform using pH Sensitive Block Copolymers***,* ***L.E. Brown****,* The University of Sheffield, UK*, S.L. McArthur,* Swinburne University of Technology, Australia*, G. Battaglia, P.C. Wright*, The University of Sheffield, UK

The development of pH sensitive, biocompatible block copolymer vesicles has enabled the intracellular delivery of water soluble drugs and proteins. Improving the encapsulation efficiency of the vesicles is now a critical parameter. Transferring the production method to a microfluidic device creates the potential to vary the encapsulation conditions and improve this efficiency. In this work, a flow focussing microfluidic device is used. The self assembly of PMPC-b-PDPA block copolymer vesicles is induced within the device by changing the pH of the flows within the microchannels. The use of pH shift eliminates the need for organic solvents currently required for glass capillary production methods. This enables the biocompatibility of the block copolymers to be maintained, an essential factor for their application as molecular delivery vehicles.

The flow focussing microfluidic device was produced through standard soft lithography techniques. A three-channel flow system is used with the copolymer in solution at pH6 in the central channel and aqueous buffered solutions flowing in the channels either side. The laminar flow conditions within the microfluidic device result in a pH gradient at the interfaces where the three channels meet and where the block copolymers self-assemble into vesicles. These vesicle formation processes have been imaged using confocal microscopy via FRET with a block copolymer containing both rhodamine and fluorescein isothiocyanate groups. Dynamic light scattering and TEM were used to confirm vesicle formation.

With 50nm to 250nm vesicles continuously being produced within the device it was then possible to investigate whether higher encapsulation efficiencies can be achieved using the microfluidic device. The protein myoglobin was introduced through the central channel along with the copolymer. Spectrophotometric analysis indicated the overall the efficiency of the encapsulation process within the device is not a significant improvement on the standard bulk methods currently used, involving sonication of the vesicle solution containing the molecule to be encapsulated. Despite this, the continuous nature of microfluidic devices, as well as the lack of organic solvents being used in the production process indicates that the development of these devices offers a viable alternative production method for polymer vesicles that may enable the increases in encapsulation efficiency to be achieved. Work is ongoing to achieve this using the same pH shift mechanism within a glass capillary microfluidic device.

3:40pm **BM+MN+MS+TF+BI-ThA6 Integration of a Microfluidic Flow Cell Array with SPR Microscopy for In Situ Microarray Formation and Biomolecule Interaction Analysis***, J. Liu, M. Eddings,* University of Utah*, A. Miles,* Wasatch Microfluidics*, B. Gale,* ***J. Shumaker-Parry***, University of Utah

Analysis of biomolecule interactions based on surface plasmon resonance (SPR) microscopy provides a label-free approach to monitoring arrays of biomolecule interactions in real time. Typically the microarray sensing surface for these measurements is prepared ex situ and a single or few channel flow cell is used for the biomolecule interaction studies. The multiplexing nature then is derived from the microarray and the number of samples that can be run simultaneously is rather limited, diminishing the potential application for assays requiring a high-throughput approach due to a large number of samples. One example of this is the need to monitor for anti-drug antibodies from a large pool of patient samples during clinical trials of biotherapeutics. We demonstrate the capability of a multi-channel microfluidic flow cell array (MFCA) to expand the throughput capability when integrated with SPR microscopy. In addition, the MFCA provides an in situ approach to array fabrication that allows full characterization of the biomolecule immobilization process. We use the MFCA for delivery of sample solutions with continuous flow in 48 channels in parallel for rapid microarray creation and binding analysis while using SPR microscopy for real-time monitoring of these processes. Label-free measurement of antibody-antibody interactions demonstrates the capabilities of the integrated MFCA-SPR microscopy system and establishes the first steps of the development of a high-throughput, label-free immunogenicity assay. We demonstrate a limit of detection (LOD) of ~ 80 ng/ml for the particular antibody pair we studied. This LOD is ~6 times lower than the industry recommended immunogenicity assay detection limit. The high-throughput nature of the integrated system allows a large number of replicate experiments, including control experiments, to be performed simultaneously on the same sensor surface in a short time. The integrated system also will be applicable for more general high-throughput protein-array based analysis.

4:20pm **BM+MN+MS+TF+BI-ThA8 Nanochannel Stretching of Nucleic Acids: Towards Epigenetic Analysis***, D.E. Streng, S.-F. Lim, A. Karpusenka, J. Pan, J.A. Hook,* ***R. Riehn***, NC State University

Nanochannels with a diameter of about 100nm2 are a novel method for stretching DNA for genomic investigations. Such devices are implemented through standard nanolithography in fused silica. The elongation of DNA results from an interplay of steric and entropic effects. Previous applications of nanochannel stretching included sizing, restriction mapping, and observation of transcription factor binding.

We show here that nanochannels can also be used to map the site-specific epigenetic state of DNA. In particular, we show here that the concept by nanoconfinement can be extended to chromatin, or DNA complexed to histones, and that the stretching is within the range expected from the de Gennes theory. We also demonstrate that the location-resolved cytidine methylation state of DNA can be mapped by specific fluorescent labeling. We will discuss the basic operation of these technique, and the application to artificial substrates with predefined epigenetic marks.

4:40pm **BM+MN+MS+TF+BI-ThA9 Microfluidic Models of Endothelial Cell Sprouting in Response to Biomechanical and Biochemical Microenvironments***,* ***A.M. Shamloo****, S.C. Heilshorn*, Stanford University

A novel microfluidic device was designed in order to generate stable, quantifiable concentration gradients of biomolecules in a cell culture chamber for 2-D and 3-D studies of shear-sensitive cell types such as endothelial cells. Endothelial cells form the inner lining of blood vessels and initiate a critical step in angiogenesis (the sprouting of new blood vessels) during wound healing and cancerous tumor growth. Therefore, a deeper understanding of the critical biomechanical and biochemical factors regulating endothelial cell sprouting can lead to improved clinical therapies for a multitude of diseases. Concentration distribution of soluble growth factors inside the microfluidic cell culture chamber was determined by simulation and experiment, and the stability of the gradient was verified over multiple hours. This device allows independent tuning of the matrix rigidity, the growth factor absolute concentration, and the growth factor concentration gradient steepness within a single experimental platform.

Sprout formation of dermal microvascular endothelial cells was studied within collagen gels of varying density (0.3 - 2.7 mg/mL, corresponding to shear moduli of 8 – 800 Pa) that contained stable gradients of soluble vascular endothelial growth factor (VEGF). These experiments revealed that endothelial sprouting into multi-cellular, capillary-like structures is optimized at an intermediate collagen matrix density (G'~100 Pa). At lower matrix densities, cells were more likely to lose their coordinated motion and migrate as individual cells through the matrix; while at higher matrix densities, the cells formed broad cell clusters that rarely elongated into a sprout. Sprout thickness directly correlated with matrix rigidity, with thicker and less frequent sprouts present in gels with the highest shear moduli. Intriguingly, our 3D experiments also found that endothelial sprouts alter their sensitivity to VEGF depending on the matrix density, suggesting a complex interplay between biochemical and biomechanical factors. As matrix stiffness increases, steeper VEGF gradients and higher VEGF absolute concentrations are required to induce directional sprouting. In more compliant gels, endothelial sprouts that originally misaligned were able to turn and properly reorient parallel to the VEGF gradient; however, this turning phenomenon was only rarely observed in stiffer gels. These results demonstrate that matrix stiffness is an effective factor in stabilization and orientation of endothelial cells during sprouting and suggests new anti-angiogenic strategies for potential cancer treatment as well as pro-angiogenic strategies for regenerative medicine scaffolds.

5:00pm **BM+MN+MS+TF+BI-ThA10 Plasma Polymerisation of PDMS for Microfluidic Applications**, ***S. Forster***, *A.G. Pereira-Medrano, G. Battaglia, P.C. Wright,* University of Sheffield, UK, *S.L. McArthur*, Swinburne University of Technology, Australia

Polydimethylsiloxane (PDMS) has become the most popular material choice for a wide range of microfluidic bioengineering applications, including proteomics, protein separations and drug discovery and development. The reasons its popularity lie mainly in its highly advantageous fabrication requirements when compared to traditional materials such as glass and silicon. However, PDMS has some fundamental drawbacks, namely a lack of functionality present at the surface, high protein fouling and an inability to retain stable surface modification due to its motile hydrophobic monomer. These factors can lead to the loss of specificity and sensitivity in many bioassays. Due to this reason much work has been completed looking into surface modification of PDMS for such applications. Here an alternative method of stable surface modification of PDMS for many microfluidic applications through enhanced curing conditions and plasma polymerisation is shown. Stable and functional surface coatings have been achieved on bulk PDMS and within microfluidic channels. Bulk surfaces were characterised using a combination of XPS and ToF-SIMS, while coated micro-channels were tested using confocal microscopy and various assays. This methodology has been used in many applications and one area where it has proven extremely useful is in microfluidic proteomics where surface properties are of paramount importance due to the inherently small volumes and quantities associated with biological samples. Firstly, plasma polymer coated PDMS micro-channels were utilised for on-chip IEF protein separations (i.e. separating proteins bases on charge) and showed reduced electrosmotic flow (EOF) and protein adsorption within the device. Secondly, a µIMER (micro-immobilised enzyme reactor) was produced using plasma polymer coated PDMS devices. The µIMER was then used in 'shotgun' protein digestion applications in conjunction with Mass Spectrometry where it was shown to have numerous advantages over untreated PDMS devices, as well as comparing favorably to published work on other µIMER systems. The device was used to digest single and multiple protein samples as well as complex membrane protein samples. Finally, successful covalent bonding of plasma polymer coated devices has led to the completion of polymer vesicle immobilisation within a microfluidic channel. Initial work looking at the immobilisation of polymer vesicles with an encapsulated digestive enzyme has shown to increase proteomic digestion efficiency. This success opens up the possibility of translating this technique into many potential microfluidic applications through the extensive versatility of encapsulation within polymer vesicles.

Biomaterial Interfaces
Room: Hall 3 - Session BI-ThP

Biomaterial Interfaces Poster Session II (Arrays, Sensing, Micro/Nanofabrication, SPM)

BI-ThP1 Integration of Protein Microarrays and Glyconanoparticles with Surface Plasmon Resonance Imaging to Probe Carbohydrate-Protein Interactions, ***H. Wang***, *X. Wang, M. Yan*, Portland State University

Multivalent interactions between carbohydrates and proteins are attracting increasing interest because of their importance in many biological processes. We developed a strategy integrating microarrays, nanoparticles and surface plasmon resonance imaging (SPRi) to study carbohydrate-protein interactions. Protein microarrays were fabricated and carbohydrate-coated glyconanoparticles were used as multivalent probes to target carbohydrate-binding proteins. SPRi offers a real-time sensing method to investigate multiple binding events simultaneously. The chemistry, fabrication and characterization of microarray platforms will be discussed. The binding parameters and SPRi responses with regard to surface chemistry and ligand density will be presented.

BI-ThP2 Aqueous Polymer Nanografting: AFM patterning of Poly-L-Lysine on Oxide Surfaces, ***B.S. Davis***, *H.J. Conley, J.L. Knoebel, K.B. Hurd, J.N. Harb, M.R. Linford, R.C. Davis*, Brigham Young University

We present a scanning probe lithography technique that allows for patterning of adsorbed, water-soluble polymers on functionalized oxide surfaces. SiO_2 and borosilicate glass surfaces were functionalized with a negatively charged carboxyl-terminated silane monolayer. A ca. 2 nm poly-L-lysine layer was then deposited over the silane film. An atomic force microscope (AFM) probe was used to scribe away lines and areas of the positive poly-L-lysine layer, exposing the negatively charged silane underlayer. The AFM scribing experiments were performed both in air and in water. Regions were scribed and then backfilled with a fluorescently tagged polymer. Characterization of the scribing was done with tapping mode AFM. Optical fluorescence microscopy was used to image backfilled regions. AFM height and phase mode data showed lines and spaces with half pitch features as small as 12 nm created with a scribing force of 0.3 µN.

BI-ThP3 High Sensitivity Electrochemcial Immunosensor Based on Plasma Modified TiO_2/Chitosan, *R. Khan,* North East Institute of Science & Technology, India*,* ***M. Dhayal***, Centre for Cellular and Molecular Biology, India

RF plasma treated nanocrystalline hybrid chitosan/TiO_2 matrix was used to develop high sensitivity electrochemical immnunosensor. The observed interfacial charge transfer resistance (R_{CT}) and double layer capacitance (C_d) of plasma modified matrixes were decreased. An improved strength of amide I and II groups in FTIR spectra were observed which was associated with enhancement in immobilized rabbit antibodies (IgGs) on plasma altered surfaces. Electrochemically q uantitative detection of *Ochratoxin-A* (OTA) concentrations varying in buffer solution was carried out on IgGs immobilized plasma treated and untreated ITO/CS/TiO_2 electrodes. Plasma modified electrodes had showed very good sensitivity at very low OTA concentrations whereas the untreated electrodes sensitivity was deprived.

BI-ThP4 Measuring Magnetic Properties of Individual Magnetosomes by Scanning Transmission X-ray Microscopy, ***A.P. Hitchcock***, *K.P. Lam, M. Obst,* McMaster University, Canada*, U. Lins*, Universidade Federal do Rio de Janeiro, Brasil

We have studied the Fe 2p X-ray magnetic circular dichroism (XMCD) of individual magnetosomes - biomineralized ferrimagnetic nano-crystals in magnetotactic bacteria (MTB) - using scanning transmission X-ray microscopy(STXM). Magnetosomes are intracellular magnetite (Fe_3O_4) or greigite (Fe_3S_4) nano-crystals (typically 30-60 nm in size), enclosed in a lipid membrane. A chain of magnetosomes is used by MTBs to orient relative to the earth's field, and guide motion to optimal living environments. Our initial goal, which has been achieved, was to demonstrate that the STXM has the capability to investigate magnetic properties of sub-50 nm areas in biological systems. The Fe 2p XMCD of individual Fe_3O_4 magnetosomes of MV-1, a marine vibrio species of magnetotactic bacteria, was measured with the sample at 30 degrees relative to the beam to sense the in-plane magnetic component. This is the first such measurement of the XMCD of a single magnetosome to our knowledge. Evidence for multiple domains was found in some magnetosomes. In addition we have begun to explore the associated biochemistry by STXM spectromicroscopy at high spatial resolution (30 nm) at the C 1s and O 1s absorption edges. The combined XMCD and biochemical imaging will help further the understanding of biomineralization processes present in MTB and other environmental organisms.

Research funded by NSERC. The Canadian Light Source is supported by NSERC, NRC, CIHR, and the University of Saskatchewan. Some measurements were also made at STXM 11.0.2 at the Advanced Light Source, which is supported by the Division of Basic Energy Sciences of the U.S. Department of Energy.

BI-ThP6 A Novel Technique for the Determination of Orientational Effects in Bio-Composite Materials*, D. Ziskind, T. Geron, S. Fleischer, K. Zhang,* ***S.R. Cohen***, *H.D. Wagner*, Weizmann Institute of Science, Israel

Dentin is a natural composite material consisting of highly mineralized tubules (peritubular dentin, PTD) embedded in an intertubular matrix (ITD) consisting predominantly of collagen. Although the mechanics of dentin has been studied for over a century, only recently has the advent of nanomechanical testing allowed investigation of its microscopic characteristics. In particular, the role of the PTD in overall dentin mechanics can now be explored. By selecting small enough volumes of dentin, the orientational effect of the tubules can be examined. In this study, micron-sized pillars were fashioned by a novel femtosecond laser ablation technique, which avoids the material damage induced when milling is performed by a high energy ion beam or ablation by slower laser pulses. Testing of these structures was performed in a nanoindenter by recording force vs. deformation curves under constant strain rate (until failure) while compressing the pillar with a flat punch tip. These data provided both the modulus and strength of the sample. The small size of the pillars, approximately 20 x 20 microns in cross-section and 100 microns height, guarantee that the tubular orientation is well-defined within a single pillar compression experiment. A statistical correlation was observed between the tubule orientation and measured modulus, with a higher modulus being recorded when the tubule axis was oriented along or near the axis of compression. Such studies allow correlations between the local tubular orientation and biomechanical function. The new method described in this work does not expose the sample to dehydration in vacuum or high energy ions, and does not require coating with conductive materials. It is generally applicable to a variety of biological specimens.

BI-ThP8 Characterizing the Carbohydrate Microarray: XPS, ToF-SIMS, SPR, and ELLA Analysis of Glycan-Modified Surfaces, ***F. Cheng***, *M. Dubey, H. Nguyen, S. Jing, J. Burk-Rafel,* University of Washington*, M. Dhayal,* Centre for Cellular and Molecular Biology, India*, D. Ratner*, University of Washington

Self-assembled Monolayers (SAMs), especially alkanethiols on gold, have been extensively used as model system for studying surface modification strategies. In this work, we utilize this platform to fabricate carbohydrate-modified biosensors composed of mixed monolayers of mannose headgroups and oligo(ethylene glycol) (OEG) moieties on gold. We have extensively used x-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), surface plasmon resonance (SPR), and enzyme-linked lectin assay (ELLA) to understand the composition, structure and reactivity of these mixed SAMs to carbohydrate-binding proteins (lectins). XPS and ToF-SIMS results give a clear indication that the composition of mannose on the surface is directly proportional to its molar ratio in solution. However, when these sensors are exposed to lectins, XPS shows that the amount of protein is inversely proportional to the amount of mannose present on the surface. We performed SPR studies to obtain a quantitative comparison of the amount and multivalent binding of lectins on these mixed SAMs. Detailed study of this system using XPS, ToF-SIMS, SPR and ELLA suggests that an optimum density of mannose on the surface is required to improve the sensitivity and stability of these sensors.

BI-ThP9 Interferometric Lithography of Self-Assembled Monolayers, ***J. Adams***, *G. Tizazu,* University of Sheffield, UK, *S.R.J. Brueck,* University of New Mexico*, G.J. Leggett,* University of Sheffield, UK*, G.P. Lopez*, University of New Mexico

Self-assembled monolayers (SAMs) have proved to be highly versatile systems for molecular patterning. Previous work using near-field optical methods has demonstrated that exceptional spatial resolution may be achieved using SAM resists, by exploiting the fact that the photosensitive group is confined to a monomolecular film adsorbed to a solid surface. However, near-field methods are serial in nature. Here we have exploited interferometric approaches in combination with SAM resists to fabricate a variety of nanostructured materials. Extremely high resolution has been achieved by exploiting the monolayer nature of the resist. Interferometric

lithography (IL) is rapid, and uses minimal instrumentation. IL using a cw, 244 nm frequency doubled Ar-ion laser source has been found to yield structures as small as 35 nm using SAM resists, over macroscopically extended areas. SAMs of alkanethiols on gold may be photo-oxidized to yield weakly bound sulfonates that may be displaced by solution-phase adsorbates to yield patterns of chemical composition. Here, we demonstrate the fabrication of patterns of surface free energy with a period of 200 nm. Protein adsorption may be controlled by using IL to selectively photodegrade oligo(ethylene oxide) (OEG) terminated SAMs of alkanethiols on gold and of trichlorosiloxanes on glass. Nanopatterned streptavidin formed this way retains its ability to bind biotinylated proteins. Finally, monolayers of phosphonic acids on titanium dioxide may be readily patterned and used as templates for the fabrication of a variety of architectures, including 35 nm TiO2 structures on glass. IL is an inexpensive, fast and convenient means of producing molecular nanostructures over square centimetre and larger areas.

BI-ThP10 Absolute Quantification of Bio-molecules Immobilized on Self-Assembled Monolayers, ***H. Min**, Y.J. Lee, D.W. Moon, T.G. Lee*, KRISS, Rep. of Korea

Biochips such as DNA and protein chips are becoming increasingly important in molecular diagnostics due to their low cost and the need for automated and easy-to-handle techniques. However, only a fraction of biochip products are approved by the FDA for clinical purposes because of the demand for accurate and reproducible biochip performance that can also be quantified. In this study, we develop a new method for the absolute quantification of the probe molecules (DNA, PNA and protein) immobilized on self-assembled monolayers (SAMs) by using medium energy ion scattering (MEIS) spectroscopy. In addition, measuring the amounts of target molecules in interaction with probe molecules on biochip surfaces, we determined the hybridization efficiencies of the DNA-DNA and PNA-DNA systems or the interaction efficiency of the protein-protein system. Our results show that this new methodology would be very useful for quality control of biochips in bio-medical applications.

BI-ThP11 Optically Responsive Nanoparticle Layers for the Label-Free Readout of High-Density Peptide Libraries, ***R. Dahint**, F.C. Liu, N. Waly, H.O. Guvenc,* University of Heidelberg, Germany, *T. Felgenhauer, F. Breitling,* German Cancer Research Center, Germany, *M. Himmelhaus*, University of Heidelberg, Germany

Recently, we developed a novel complex material with combined optical and biological functionality [1, 2]. It consists of dielectric nanoparticles, which are adsorbed onto a plain gold surface and subsequently metallized by deposition of gold colloid prior to electroless plating. Upon reflection of white light, the layers exhibit pronounced extinction peaks which shift to higher wavelengths when molecules adsorb onto the surface. For simple alkanethiols a significantly higher red-shift of the extinction maximum was observed than reported for conventional surface plasmon resonance. To detect biomolecular interactions in array format it is crucial to guarantee homogenous optical response of the nanoparticle layers on macroscopic scales. We will, therefore, discuss the impact of different coating procedures on the optical properties of the films. To optimize sensitivity, effects of particle layer density, dielectric interlayers and plating time have been investigated. We also compared the response of core-shell nanoparticle layers to the optical properties of surface adsorbed gold colloid films. The final goal is to incorporate high-density peptide arrays into the optically responsive nanoparticle films by combinatorial laser printer synthesis [3] to facilitate label-free high-throughput screening of biomolecular interactions for biomedical and pharmaceutical applications. For this purpose, the peptide probes are embedded into a protein resistant matrix based on poly(ethylene glycol)methacrylate (PEGMA). The stability of both nanoparticle layers and PEGMA coating has been optimized with respect to the chemical and physical requirements of the biomolecular coupling reactions.

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BI-ThP12 Enzymatic DNA Polymerization: Potential as Signal Amplification, ***V. Tjong**,* Duke Univerisity, *A. Hucknall,* Duke Univesity, *H. Yu, A. Chilkoti*, Duke University

We have developed a new technique for on-chip, isothermal signal amplification using 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 incorporate non-natural fluorescent dNTPs into a long polymer chain of single stranded DNA (ssDNA). We quantified the TdT mediated signal amplification on the surface by immobilizing ssDNA oligomers on a glass surface followed by surface initiated enzymatic polymerization of DNA. We examined the effect of the concentration of the different natural dNTPs, and the molar ratio of fluorescent dNTPs to natural dNTPs on the length of the polymerized DNA strand and the degree of fluorophore incorporation. These experiments allowed us to optimize the polymerization conditions to incorporate a large number of fluorescent nucleotides (up to ~100 fluorescent dNTP/chain) into the ssDNA chain catalyzed by TdT. For Cy3-labeled dATP, this translated to a maximum of ~40 fold signal amplification through the incorporation of multiple fluorophores into the extended DNA chain. This methodology has the attractive attributes that it is both isothermal and on-chip –as the fluorophores are covalently incorporated into a ssDNA chain that is grown from a tethered DNA strand at 37 ºC. We anticipate the use of this amplification modality for the development of sandwich fluoro-immunoassays and DNA microarrays where binding of the detection Ab in a sandwich assay or the target strand in a DNA microarray provides the 3'-OH groups necessary to initiate on-chip fluorescence amplification of the binding event.

BI-ThP13 Surface Characterization of Reactive Surface Patterns and Their Selective Bio-immobilization Reactions, ***F. Liu**,* University of Utah, *M. Dubey,* University of Washington, *K. Emoto,* Accelr8 Technology Corporation, *H. Takahashi, D.W. Grainger,* University of Utah, *D.G. Castner*, University of Washington

Surface patterning is often used to immobilize bioactive molecules including proteins, oligonucleotides and small ligands, to localize surface reactions for bioassays and to provide desired cell and bacterial adhesion. This study reports extensive surface analysis of a commercial PEG-based surface chemistry with active ester (NHS)-activity in patterned films. The study followed sequential immobilization and masking reactions on photolithographic patterns used to immobilize peptides, proteins, and cultured cells to specific patterned regions of NHS-reactive or de-activated chemistry.[1] Biotin and peptide patterns were correlated to patterned reactive NHS surface chemistry using high-resolution time-of-flight secondary ion mass spectrometry (ToF-SIMS) for each species. Cell growth and patterning in 15-day serum cultures followed peptide patterns. In other patterned samples, mixed protein (streptavidin and HaloTag™) solutions produced spontaneous self-recognized, bound patterns on photolithographically surface-patterned affinity ligands for each (i.e., biotin and chloroalkane, respectively). The approach uses high-affinity protein-surface self-selection onto patterned PEG-NHS surfaces that exhibit intrinsically low non-specific adsorption background. Fluorescence images and ToF-SIMs imaging of the resulting protein surface selection from mixtures support highly specific interactions of proteins with their respective ligands patterned on the surface.[2] On-going work comparing ToF-SIMs imaging of antibody F_c and F_{ab} fragments supports some ability to produce different whole antibody orientations on neighboring patterns spontaneously. Use of principal component analysis (PCA) helps to increase the ToF-SIMS image contrast and provide protein orientational details based on amino acid compositions.[3]

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[2]Dubey M., Emoto K., Takahashi H., Castner D.G., Grainger, D.W., submitted, 2009.

[3] Dubey, M., Takahashi, H., Lew, F., Emoto, K., Castner, D.G., Grainger, D.W., submitted, 2009.

BI-ThP14 Temperature-Induced Conformational Changes of Antifreeze Proteins in Aqueous Solution via Overlayer-Enhanced Attentuated Total Reflectance Fourier Transform Infrared Spectroscopy (OE-ATR-FTIR), ***T. Ng**, D.P. Land,* University of California, Davis, *X. Wen*, California State University, Los Angeles

Protein structural studies are commonly performed using techniques such as X-ray diffraction and Nuclear Magnetic Resonance (NMR). Unfortunately, these techniques make it difficult to study proteins in their native environment. Studies on protein structures via FTIR are often done with high protein concentration or in deuterated solvent. Tethering a protein near the interface of a gold-coated germanium internal reflection element (IRE) concentrates the protein near the interface and allows one to detect the protein with an increased signal-to-noise. By analyzing the amide I spectral region of the protein, the secondary structure of the protein can be determined and any conformational changes in these structures can be monitored. The secondary structure of an antifreeze protein extracted from *Dendroides canadensis* is determined in the aqueous and frozen states and

show a decrease in the amount of beta sheet structures and an increase in the amount of turn structures upon freezing.

BI-ThP15 Development of Electric-Field Nanolithography for Selective Surface Modification of Non Bio-Fouling Surface Coatings, ***R. Ferris***, Duke University

Though surface coatings of Poly-Ethylene Glycol (PEG) has been recognised for decades as a particularly effective non-fouling surface, recent advances in polymer brush fabricated thin film Poly-Oligio(Ethylene Glycol) Methyl Methacrylate (POEGMA) has presented a myriad of novel applications. The capability to easily tune brush height and still maintain a high surface grafting density has been shown to prepare surfaces which essentially eliminate the non-specific adsorption of both proteins and cells. Here we present the effects of selectively modifying the surface of polymer brush surfaces, such as POEGMA, via Electric Field Nanolithography (EFN).

EFN, utilizing a spatially localized potential bias to produce chemical modifications sites on a wide range of surfaces, has proven capable of serially modifying the chemical and conformational structure of a variety of polymer-brush film surfaces such as POEGMA, PolyAcrylic Acid, and PolyMethyl Methacrylate surfaces. Such work, however, has presented an interesting and novel bias voltage dependence previously unreported in literature.

Traditionally, EFN has been utilized to produce oxide-rich regions available for further reaction sites processing. Integration of a responsive, non-fouling, polymer brush surface, however, severely alters the voltage modification dependence from the traditional negative tip bias requirement to the now positive tip bias dependence.

Each polymer thin film studied presents a different surface energy landscape, hydrodynamic interaction characteristic, and intramolecular interaction. Presented results, in addition to the contrasted effects seen on spun-coated polymer thin films, will further illuminate the mechanism and effects of EFN integration with polymer brush thin films. In addition to topographical and chemical effects of these thin films, an elevated, film-thickness dependent, threshold bias voltage is reported. Films have been characterized using Xray Photonelectron Spectroscopy, Atomic Force Microscopy, and Contact angle measurements.

In furthering the understanding of how EFN interacts with polymer thin films, it will become possible to produce selective deposition of biological arrays and assays for next generation sensing applications.

BI-ThP16 Template-stripped PZT Thin Films as Substrates for Charge Assisted Assembly of Biological and Organic Molecules, ***R.E. Ducker***, *R.T. Hill, A. Chilkoti, B.B. Yellen, S. Zauscher*, Duke University

Ferroelectric thin films, such as Lead zirconium titanate (PZT) have attracted a great deal of interest in recent years due to their piezoelectric and ferroelectric properties. The applications for these films are typically in microelectromechanical systems (MEMS) and ferroelectric non-volatile random access memories. Recently a technique called ferroelectric lithography (FL) has been developed, which can be used to make charged features on surfaces of ferroelectric materials. FL is used to create polarization patterns by applying a voltage to the surface of a ferroelectric material (such as PZT) via an atomic force microscopy (AFM). To achieve this, a DC voltage is applied between the tip and a Pt electrode on the bottom of the PZT to achieve features ranging from 100s of nanometers to several microns. The characterization of the polarization features is done using scanning Kelvin probe microscopy (SKPM). This technique can confirm the presence of an out-of-plane polarization component due to the reorientation of the ferroelectric domains in the PZT. The polarized domains can then be used to direct the assembly of charged entities. To date only inorganic species and gas phase organic molecules has been reported. Here we show the assembly of biological and organic molecules from the liquid phase.

To fabricate these structures an improved method of fabricating PZT thin films with a low roughness is presented. Sol-gel deposition is a relatively cheap and easy method of preparing thin films of PZT. However, films produced in this way can exhibit very high roughness which can make them incompatible with studies involving AFM. We use a template-stripping method to produce extremely flat PZT surfaces. These flat surfaces used in-conjunction with the FL (as described above), can be used to study the directed assembly of charged species on the surface of the PZT using standard AFM techniques. To demonstrate this, the directed assembly of polyelectrolyte layers and proteins on the surface of the PZT is shown.

BI-ThP17 Nano-Dispersion of Ferulic Acid, ***A.S. Madani***, *F. Pourmorad*, Pharmaceutical Research Center, Iran

Using natural compounds such as flavonoids in various diseases are under special consideration. Unfortunately poor solubility of flavonoids is an important limitation in preparing pharmaceutical dosage forms. It is reported that preparing nonoparticles can overcome poor solubility problem of the mentioned compounds. The O/ W nano dispersions are a group of preparations in which the poorly soluble drugs could be dissolved in the oil core and/or adsorbed on the O/W interface. Dispersion of flavonoids was prepared by solvent diffusion method in organic phase and lecithine. After adding the dispersion to the aqueous phase, ultrasonication and ultra centrifugation were carried out. A mixture of tween 80 and water were added to the mixture and then ultrasonicated again. Solvent was removed under reduced pressure at 50° C using nitrogen-purged vials. The particles were then evaluated for size and size distribution, zeta potential (ZEN 3000, Malvern, England), shape, percent entrapment, and in vitro release. Differential scanning calorimetry method was used to understand the thermotropic properties and phase transition behavior. Droplet size of nanoparticles of ferulic acid was 108 ± 12 nm.

BI-ThP18 Probing Orientation of Immobilized Humanized Anti-Lysozyme Variable Fragment by Time-of-Flight Secondary Ion Mass Spectrometry, ***J.E. Baio***, *F. Chen, L.J. Gamble, D.G. Castner*, University of Washington

The ability to orient proteins on surfaces to control exposure of their biologically active sites benefits a wide range of applications including protein microarrays, antibody-based diagnostics, affinity chromatography, and biomaterials that present ligands to bind cell receptors. As methods to orient proteins are developed, techniques must also be developed that provide an accurate characterization of immobilized protein orientation. In this study, x-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) were used to probe the orientation of a surface immobilized variant of humanized anti-lysozyme variable fragment (HuLys Fv, 26kDa). This protein contained both a His-tag and a cysteine residue, introduced at opposite ends of the HuLys Fv. Previously, we have shown that we could successful control orientation of a Protein G fragment via a cysteine-maleimide bond. To induce opposite end-on orientations of the HuLys Fv variant, it was immobilized onto maleimide oligo(ethylene glycol) (MEG) and nitrilotriacetic acid (NTA) terminated substrates. The thiol group on the cysteine residue will selectively bind to the MEG groups, while the His-tag will selectively bind to the Ni loaded NTA groups. Protein coverage, on both surfaces, was monitored by the change in the atomic % of N, as observed by XPS. The height of the immobilized protein (3nm) was larger than the typical sampling depth of ToF-SIMS, consequently it only samples the top portion of the protein. This was confirmed by principal component analysis (PCA) of the ToF-SIMS results, which demonstrated a clear separation between the two samples based on the intensity differences of secondary ions stemming from amino acids located asymmetrically in HuLys Fv (Histidine: 81, 82, and 110 m/z; Phenylalanine: 120 and 131 m/z). For a more quantitative examination of orientation, we developed a ratio comparing the sum of the intensities of secondary ions stemming from the histidine and phenylalanine residues at either end of the protein. The three-fold increase in this ratio, observed between the MEG and NTA substrates, indicated opposite orientations of the HuLys Fv fragment on the two different surfaces.

BI-ThP19 Biomimetic Metallic Electrodes for Intracellular Electrical Measurements, ***P. Verma***, *N. Melosh*, Stanford University

Interfacing living matter to electronics with the ability to monitor and deliver spatio-temporal signals to cells or cell networks is promising for various fundamental biophysical studies and also for applications such as high resolution neural prosthetics, on-chip electrically addressed artificial neural networks and arrayed on chip patch-clamps. Developing an inorganic nanostructure that can specifically and non-destructively incorporate into biological membranes is the key to such an interface. We report an approach towards this interface by functionalizing a nanoscale metallic post to mimic a transmembrane protein to directly insert into the lipid membrane and form a tight seal. These post-electrodes were formed by evaporation and lift-off onto conductive bottom electrodes, with 5-10 nm thick hydrophobic bands around the edge of the post formed by molecular self assembly. We recently reported AFM measurements of these posts inserting into lipid bilayers and showed that different molecular functionalizations adhered within the hydrophobic lipid core with different strengths depending on their molecular mobility. Here we describe nanoscale electrical measurements with these post-electrodes on red blood cells to determine the leakage current at the electrode-membrane interface.

BI-ThP20 An Alternative Solution Based Approach to PTCDI-Melamine Network Fabrication on Au(111), ***V. Korolkov***, *N. Haggerty, M. Blunt, S. Allen, C.J. Roberts, S.J.B. Tendler*, University of Nottingham, UK

Two dimensional (2D)-controlled adsorption is a versatile tool for creating well-defined arrays of biological molecules on surfaces. Such surfaces hold potential for a wide range of future applications, including for the development of biosensors and biomolecular screening technologies. Functionalizing a surface with some periodical structure (or a network) is one promising way to spatially control the adsorption process. Hydrogen-bonded networks are reported to be well-ordered structures, presenting periodical 2D-pores suitable for the adsorption of different guest molecules, and thus may provide a reasonable template for 2D-controlled biomolecular adsorption.

Up to the present, only a few studies have focused on solution based approaches to the fabrication of H-bonded networks, and so in this study we have concentrated our efforts towards optimizing a solution based preparation procedure for perylene tetracarboxylic diimide (PTCDI)-melamine network from dimethylsulfoxide solution. Investigations of the stability of this network over different parameters, led us to a useful, reproducible technique for creation of PTCDI-melamine network over a large surface area. It was shown that temperature plays a crucial role in ordering of PTCDI and melamine molecules on the surface.

Optimal conditions for oligonucleotide adsorption into the network-pores were also determined. This work employed several complementary surface analytical techniques to image the network structure (STM, AFM) and the controlled deposition of biomolecules (AFM, XPS).

Biomaterial Interfaces
Room: K - Session BI+AS+NS-FrM

Micro and Nanoengineering of Biointerfaces II
Moderator: E.O. Reimhult, ETH Zurich, Switzerland

8:20am **BI+AS+NS-FrM1 Colloid Crystal Surface Patterning for Studying Biointerface Phenomena**, ***P. Kingshott***, Aarhus University, Denmark **INVITED**

Patterning of many types of biomolecules over length scales ranging from micrometers to nanometers is of great interest for biosensors, cell culture dishes, medical implants and tissue engineering. Ideally these devices require attachment of biomolecules at specific locations on solid substrates with precisely controlled chemistry, but to function fully the non-specific adsorption in surrounding regions must be prevented. Currently, the most widely used techniques for patterning are photolithography, soft lithography and electron beam lithography, all of which involve multi-step surface modification directly onto substrates, and are time consuming and expensive. We have shown recently that highly ordered binary colloid patterns, with controllable dimensions, can be generated from simple self-assembly of large and small particles onto surfaces, where single layers of large particles are surrounded by crystals of smaller particles. In addition, when the particles are pre-coated with proteins (e.g. albumin, lysozyme and antibodies) the assembly process also takes place. This opens up the possibility of patterning many proteins on one substrate with controllable dimensions and high order. The crystals are also used to generate chemical patterns since the large particles act as a mask during, for example sputtering of Au, since the region in contact with the substrate remains uncoated. The thickness of gold features can be controlled by the sputtering time. We demonstrate that the resultant Au layer can be coated with a protein resistant mercapto-oligo(ethylene glycol) layer ((1-mercapto-11-undecyl)-tri(ethylene glycol)) that allows selective adsorption of fluorescently labelled proteins, such as FITC-labelled antibodies or rhodamine-labelled albumin, only onto the Si regions of the pattern. In another approach, binary patterns made from silica and amine polystyrene particles are heated at 100 ºC (above glass transition temperature of polystyrene) followed by etching with HF to remove the silica particles creating highly ordered 2- and 3D porous substrates. In summary, we introduce a novel method for generating highly-ordered patterns from colloid crystals that is very fast, inexpensive, and allows patterns of multiple biomolecules over large areas in 2- and 3D.

9:00am **BI+AS+NS-FrM3 Biological Nanoarrays: from Protein-DNA Interaction Studies to Cell Adhesion Investigations**, ***M. Palma***, *J. Abramson, M. Schvartzmann, A. Gorodetsky, C. Nuckolls, M.P. Sheetz, J. Hone, S.J. Wind*, Columbia University

Nanopatterned arrays of biomolecules are a powerful tool to address fundamental issues in many areas of biology.

Combining nanolithography and biomolecular self-assembly strategies, we report on the fabrication of nanopatterned biomimetic surfaces and their use in a variety of biological studies.

We have fabricated arrays of Au/Pd nano-dots of dimensions down to the sub-10nm regime using electron-beam and nanoimprint lithography. Different chemical strategies at surfaces have been pursued to organize biological relevant nanoarchitectures into hierarchical arrays in which structural parameters, such as the spacing and nature of specific functional groups, could be systematically varied and controlled.

The generation of DNA nano-dot arrays allowed us to follow the activity (at surfaces) of a restriction enzyme in real time and at the nanoscale: fluorescence microscopy enabled the monitoring of the kinetics of such protein-DNA interaction.

Furthermore we will show how our nanopatterned biomimetic surfaces can be used to probe the importance of both the geometric arrangement (i.e. spatial ordering of transmembrane proteins, integrins) as well as the role played by peptide sequences as cell binding domains in the formation of cell focal adhesions.

Finally, we will highlight the broader utility and application of such functional nanopatterned surfaces for nanoscopic control and studies: biochemical specificity can be used to selectively place individual nanocomponents with a high degree of control over both position and orientation, as well as to organize functional nanostructures into dense arrays with very fine pitch.

9:20am **BI+AS+NS-FrM4 Arbitrary Topographical Patterns Fabrication by using Two-Photon Photopolymerization**, ***H.J. Jeon***, University of California, Berkeley, *H. Hidai*, Tokyo Institute of Technology, Japan, *D.J. Hwang, K.E. Healy, C.P. Grigoropoulos*, University of California, Berkeley

Two photon photopolymerization (TPP) is a direct laser writing technique, which is known as a powerful tool to make arbitrary 3D structures. Here we demonstrate a method for fabricating high aspect ratio (~10) patterns of varying height by using TPP process in order to study contact guidance of cells. Ridge patterns of various heights and widths were fabricated through single laser scanning steps by low numerical aperture optics, hence at much higher processing throughput. Fibroblast cells were seeded on parallel line patterns of different height (~1.5-µm, ~0.8-µm, and ~0.5-µm) and orthogonal mesh patterns (~8-µm and ~4-µm height, ~5-µm and ~5.5-µm height, and ~5-µm and ~6-µm height). Cells experienced different strength of contact guidance depending on the ridge height. Furthermore, cell morphology and motility on microscale anisotropic cross patterns and parallel line patterns in different aspect ratio (1:2, 1:4, and 1:∞), size of grid (12-, 16-, and 24-µm distance neighboring longer side ridges) was also studied quantitatively. The significant effect of the cross patterns on cell alignment and directionality of migration, and motility was observed on 1:4 cross patterns and parallel line patterns, even though all cross patterns could have an effect on cell attachment and morphology. Overall, it is noted that cell morphology and motility can be influenced by the height of ridges, the aspect ratio of cross pattern and the size of grid.

9:40am **BI+AS+NS-FrM5 2D & 3D Nanoarrayed Chemical Contrasts for Better Biorecognition Kinetics**, ***G.R. Marchesini***, *P. Lisboa, A. Valsesia, C. Pascual, P. Colpo, F. Rossi*, Joint Research Centre, European Commission, Italy

Monitoring biomolecular recognition events with Surface Plasmon Resonance (SPR) heavily relies on the right surface chemistry. Uniform self assembled monolayers with carboxylic functional groups are widely used but might show steric hindrance, thus limiting the interpretation of the biorecognition kinetics. Furthermore, such negatively charged surface needs to be passivated upon immobilization of the ligand to prevent nonspecific electrostatic-driven binding of components from the analyte matrix.

In the present study we evaluate alternatives based on a 2D and 3D array of carboxylic nanodomains on a chemically contrasting matrix. By means of plasma-based colloidal lithography and e-beam lithography we were able to array ≈ 200 nm wide carboxylic motifs having a hexagonal 2-D crystalline structure on a gold surface. The interstitial gold was further modified with contrasting thiol chemistries or vapour enhanced deposition of nonadhesive material like poly ethylene oxide (PEO). The two 2D nanoarrayed chemical contrasts evaluated were carboxylic nanodomains on either a methyl-based or PEO-based matrix.

In addition, the 3D nanoarray based on a carboxylated dextran hydrogel matrix was evaluated for effects on the mass transport. In these cases, mass transport is one of the major challenges when measuring binding kinetics of biointeractants on a surface using a surface plasmon resonance (SPR) biosensor. The presence of a hydrogel on the surface increases the interactant density improving the sensitivity. Nevertheless, this is done at the expense of aggravating the mass transport phenomena.

The influence of the nanoarrayed chemical contrasts combined with the sensitivity improvement due to the band-gap effect on the kinetics of model biomolecular interactants was evaluated using an imaging SPR system and correlated with surface characterization techniques as atomic force microscopy, ellipsometry, and contact angle measurements.

10:00am **BI+AS+NS-FrM6 Spatially Selective Deposition of a Zwitterion with Alkyl Pendant Groups on Periodically Poled Lithium Niobate**, ***Z.Z. Zhang***, *J. Xiao*, University of Nebraska-Lincoln, *D. Wu*, North Carolina State University, *A. Gruverman*, University of Nebraska-Lincoln, *L. Routaboul, P. Braunstein, B. Doudin*, Université Louis Pasteur Strasbourg, France, *O. Kizilkaya*, Louisiana State University, *C. Borca*, Paul Scherrer Institute, Switzerland, *P.A. Dowben*, University of Nebraska-Lincoln

We have spatially selectively deposited a zwitterion compound from the class of N-alkyldiaminoresorcinones (or 4,6-bis-dialkylaminobenzene-1,3-diones, $C_6H_2(NHR)_2(O)_2$), compounds, where R $=C_5H_{11}$. These molecules have very strong local dipoles as the delocalized benzene π molecule of the zwitterion "core" loses aromatic character due to the large charge separation. This charge separation provides this type of zwitterion molecule with a large electric dipole moment across the "benzene" like plane. Unlike the ferroelectric materials, the electric dipole of this class of zwitterions

when adsorbed on metal surface (and most substrates) is not switchable, which makes these zwitterion compounds more like an electret. We have been able to demonstrate that at least one of this class of zwitterion compound will selective adsorb from solution on periodically poled lithium niobate substrates using infra-red spectra-microscopy. The spatial localization zwitterion on lithium niobate suggests that the ferroelectric poling of lithium niobate either alters the surface chemistry of lithium niobate or that there is some dipole-dipole interaction between the substrate and the zwitterion. We believe the interaction is an interface effect as no alteration in the bulk properties has been observed from spatially resolved near edge X-ray adsorption fine structure (NEXAFS) of the bulk properties. The spatial zwitterion structure is consistent with the periodically poled lithium niobate structure. Crystals of periodically poled lithium niobate (PPLN) with congruent composition (Crystal Technologies) were used as deposition templates. A periodic domain structure (period of ~28 µm) was fabricated by depositing a photoresist mask on the +c sample face and by applying a voltage of 10 kV through a fixture with an electrolyte solution. The mask was removed after poling by means of chemical-mechanical polishing leaving behind a bare ferroelectric surface, prior to the exposure to the zwitterion molecular solution.

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Eddings, M.: BM+MN+MS+TF+BI-ThA6, 23
Emoto, K.: BI+AS+NS-ThA9, 22; BI-ThP13, 26
Eralp, T.: BI-TuM4, 6
Erickson, D.: BM+MN+MS+TF+BI-ThA1, **22**

— F —

Farkas, N.: AS2+BI-ThM10, **17**
Fears, K.: BI-TuM3, **6**
Felgenhauer, T.: BI-ThP11, 26
Ferris, R.: BI-ThP15, **27**
Filoti, D.I.: TF-ThM9, **20**
Fisher, E.R.: TF-ThM5, 19
Fleischer, S.: BI-ThP6, 25
Forster, S.: BM+MN+MS+TF+BI-ThA10, **24**
Franco, G.C.: NS+BI-MoM6, **3**
Frank, C.W.: BI-TuM10, 7; BI-TuM11, 7
Franzen, S.: TF-ThM10, 20
Franzka, S.: BI+AS+NS-ThA4, 21
Fredriksson, H.: NS+BI-MoM9, **3**
Friesen, B.A.: NS+BI-MoM10, 3
Furman, B.: NS+BI-MoM1, 2

— G —

Gale, B.: BM+MN+MS+TF+BI-ThA6, 23
Gamble, L.J.: BI-ThP18, 27; BI-TuM5, **6**; BI-TuP13, 12
Gellert, P.R.: IJ+BI+MN+SE+AS-ThM10, 18
Gellman, A.J.: NS+BI-MoM8, 3
Gerber, R.W.: TF-ThM10, 20
Geron, T.: BI-ThP6, 25
Giangregorio, M.: BI+NS-MoM1, 1
Gil, J.: BI+AS+NS-WeA11, 15
Goldsmith, C.L.: IJ+BI+MN+SE+AS-ThM5, 18
Gorodetsky, A.: BI+AS+NS-FrM3, 29
Grainger, D.W.: BI+AS+NS-ThA9, 22; BI-ThP13, 26
Greene, A.: BM+MN+MS+TF+BI-ThA3, 23
Grigoropoulos, C.P.: BI+AS+NS-FrM4, 29
Groll, M.: BI+AS+BM+MS-WeM3, **13**
Grøndahl, L.: BI-TuP11, 11
Groves, J.T.: BI+AS+NS-ThA1, **21**
Grunze, M.: BI-TuA8, 8
Gruverman, A.: BI+AS+NS-FrM6, 29
Guilak, F.: BI-TuP10, 11
Gupta, G.: BI-TuP2, 10
Guvenc, H.O.: BI-ThP11, 26

— H —

Haggerty, N.: BI-ThP20, 28
Hampel, P.: BI+AS+BM+MS-WeM11, 13
Harb, J.N.: BI-ThP2, 25
Harper, J.M.E.: TF-ThM9, 20
Hartmann, N.O.: BI+AS+NS-ThA4, 21; TF-ThM4, 19
Hasselbrink, E.: BI+AS+NS-ThA4, 21
Havenstrite, K.: BI-MoA1, **4**
Healy, K.E.: BI+AS+NS-FrM4, 29
Heilshorn, S.C.: BI-MoA6, 4; BM+MN+MS+TF+BI-ThA9, 23
Held, G.: BI-TuM4, **6**
Heydt, M.: BI-TuA8, 8
Hidai, H.: BI+AS+NS-FrM4, 29
Hill, R.T.: BI+AS+NS-WeA10, **15**; BI-ThP16, 27
Himmelhaus, M.: BI-ThP11, 26
Hipps, K.W.: NS+BI-MoM10, 3
Hitchcock, A.P.: BI-ThP4, **25**; BI-TuM6, 7
Hohman, J.N.: BI+NS-MoM2, **1**
Holmberg, M.: BI-TuA7, **8**
Homenuke, M.: BI+AS+NS-ThA3, 21
Hone, J.: BI+AS+NS-FrM3, 29
Hook, A.L.: IJ+BI+MN+SE+AS-ThM9, **18**
Höök, F.: BI+AS+NS-WeA3, 14
Hook, J.A.: BM+MN+MS+TF+BI-ThA8, 23
Hou, X.: BI-TuA7, 8
Hucknall, A.: BI-ThP12, 26
Hurd, K.B.: BI-ThP2, 25
Husale, S.: BI+AS+BM+MS-WeM12, 13
Hwang, D.J.: BI+AS+NS-FrM4, 29
Hwang, J.: AS2+BI-ThM10, 17

— I —

Isono, T.: BI-TuP8, **11**
Ista, L.K.: BI-TuP3, 10

— J —

James, C.: BM+MN+MS+TF+BI-ThA3, 23
Jankowski, A.F.: BI-TuP14, **12**
Jay, G.: BI-TuP10, 11
Jeon, H.J.: BI+AS+NS-FrM4, **29**
Jesse, S.: AS2+BI-ThM9, 17
Jing, S.: BI-ThP8, 25
Jonsson, M.: BI+AS+NS-WeA3, **14**
Jönsson, P.: BI+AS+NS-WeA3, 14
Jung, D.: BI-TuP6, 10

— K —

Kaiser, W.: BI+AS+BM+MS-WeM11, 13
Kalinin, S.V.: AS2+BI-ThM9, **17**
Käll, M.: NS+BI-MoM9, 3
Kang, H.: AS2+BI-ThM10, 17
Kao, P.: BI+AS+NS-WeA9, 15
Karpusenka, A.: BM+MN+MS+TF+BI-ThA8, 23
Kasemo, B.H.: BI+AS+NS-WeA4, 14; NS+BI-MoM9, 3

Kaufmann, S.: BI+AS+NS-ThA3, **21**; BI+AS+NS-ThA8, 22
Khan, R.: BI-ThP3, 25
Kim, K.S.: BI-TuP6, 10
Kim, M.: BI+NS-MoM2, 1
Kim, S.: BI+AS+NS-WeA11, 15
Kim, T.H.: BI+NS-MoM1, 1
King, W.: NS+BI-MoM5, 2
Kingshott, P.: BI+AS+NS-FrM1, **29**
Kizilkaya, O.: BI+AS+NS-FrM6, 29
Klingebiel, B.: TF-ThM4, 19
Knezevic, J.: BI+AS+BM+MS-WeM11, 13
Knoebel, J.L.: BI-ThP2, 25
Koh, S.J.: BI+NS-MoM6, 1; NS+BI-MoM11, 3
Korchev, Y.E.: AS2+BI-ThM1, 16
Korolkov, V.: BI-ThP20, **28**
Kubby, J.A.: IJ+BI+MN+SE+AS-ThM5, **18**
Kulp III, J.: BI-TuM3, 6
Kumar, K.: BI+AS+NS-ThA8, **22**
Kunze, A.: BI+AS+NS-WeA4, **14**

— L —

Lam, K.P.: BI-ThP4, 25
Land, D.P.: BI+AS+NS-WeA12, 15; BI-ThP14, 26
Langer, R.S.: BI-MoA3, 4; IJ+BI+MN+SE+AS-ThM9, 18
Latour, R.A.: BI-MoA10, 5; BI-TuM1, **6**; BI-TuP12, 11
Lee, S.: BI+NS-MoM5, 1
Lee, T.G.: BI-ThP10, 26; BI-TuP6, 10
Lee, W.-K.: NS+BI-MoM5, 2
Lee, Y.J.: BI-ThP10, 26
Leggett, G.J.: AS2+BI-ThM3, **16**; BI-ThP9, 25
Lemmo, A.V.: BI+AS+BM+MS-WeM5, 13
Leung, B.O.: BI-TuM6, **7**
Liedberg, B.G.: BI-TuA1, **8**
Lim, S.-F.: BM+MN+MS+TF+BI-ThA8, 23
Linford, M.R.: BI-ThP2, 25
Lins, U.: BI-ThP4, 25
Lisboa, P.: AS2+BI-ThM6, **17**; BI+AS+NS-FrM5, 29
Liu, F.: BI-ThP13, **26**
Liu, F.C.: BI-ThP11, 26
Liu, J.: BM+MN+MS+TF+BI-ThA6, 23
Lopez, G.P.: BI-ThP9, 25; BI-TuP2, 10; BI-TuP3, 10; BI-TuP7, 10
Losego, M.: TF-ThM10, **20**
Losurdo, M.: BI+NS-MoM1, 1

— M —

Ma, K.: IJ+BI+MN+SE+AS-ThM11, 18
Madani, A.S.: BI-ThP17, **27**
Mangalam, A.S.: IJ+BI+MN+SE+AS-ThM5, 18
Marchesini, G.R.: BI+AS+NS-FrM5, **29**
Maria, J.-P.: TF-ThM10, 20
Marinakos, S.: BI-TuP5, 10
Maruyama, M.: BI+AS+BM+MS-WeM11, 13
Mathieu, M.: BI+AS+NS-ThA4, **21**
Mayer, C.: BI+AS+NS-ThA4, 21
Mayer, M.: BI+AS+NS-WeA7, **14**
Mazur, U.M.: NS+BI-MoM10, **3**
McArthur, S.L.: BM+MN+MS+TF+BI-ThA10, 24; BM+MN+MS+TF+BI-ThA4, 23
McKendry, R.: AS2+BI-ThM4, 16
McKnight, T.E.: TF-ThM1, 19
Meares, C.F.: BI+AS+NS-WeA12, 15
Mei, Y.: BI-MoA3, 4
Melechko, A.V.: TF-ThM1, 19
Melosh, N.: BI+AS+NS-ThA7, 22; BI-ThP19, 27
Mendez, S.: BI-TuP3, 10
Miles, A.: BM+MN+MS+TF+BI-ThA6, 23
Min, H.: BI-ThP10, **26**
Mock, J.J.: BI+AS+NS-WeA10, 15
Moon, D.W.: BI+AS+NS-WeA11, **15**; BI-ThP10, 26; BI-TuP6, 10
Morin, E.I.: BI+NS-MoM2, 1
Music, D.: TF-ThM3, 19

— N —

Ndieyira, J.: AS2+BI-ThM4, **16**
Ng, T.: BI-ThP14, **26**
Nguyen, H.: BI-ThP8, 25
Nicolella, D.: NS+BI-MoM1, 2
Nigra, M.: NS+BI-MoM8, 3
Nikiforov, M.P.: AS2+BI-ThM9, 17
Nuckolls, C.: BI+AS+NS-FrM3, 29

— O —

Obst, M.: BI-ThP4, 25
Ogino, T.: BI-TuP8, 11
Oncins, G.: AS2+BI-ThM11, 17
Opdahl, A.: BI-TuP15, 12
Orihuela, B.: BI-TuA9, 8
Orje, J.: BI-MoA9, 5
Orlicki, J.: BI-MoA8, 4
Ovchinnikov, O.: AS2+BI-ThM9, 17

— P —

Pakizeh, T.: NS+BI-MoM9, 3
Palma, M.: BI+AS+NS-FrM3, **29**
Pan, J.: BM+MN+MS+TF+BI-ThA8, 23
Park, S.S.N.: BI+AS+NS-WeA12, 15
Pascual, C.: BI+AS+NS-FrM5, 29
Pereira-Medrano, A.G.: BM+MN+MS+TF+BI-ThA10, 24
Perez-Luna, V.: BI+NS-MoM5, 1
Persson, H.H.J.: BI+AS+BM+MS-WeM12, **13**
Petronis, S.: BI+AS+NS-WeA3, 14
Petrovykh, D.Y.: BI-TuM3, 6; BI-TuP15, **12**
Poenitzsch, V.Z.: NS+BI-MoM1, 2
Pourmorad, F.: BI-ThP17, 27
Pringsheim, E.: BI+AS+BM+MS-WeM11, 13

— R —

Rack, P.D.: TF-ThM1, 19
Ramana, C.V.: NS+BI-MoM6, 3
Rant, U.: BI+AS+BM+MS-WeM11, **13**
Ratner, D.: BI-ThP8, 25
Rawlett, A.: BI-MoA8, 4
Ray, V.: NS+BI-MoM11, 3
Redondo-Morata, L.: AS2+BI-ThM11, **17**
Reed, J.A.: BI-TuP4, **10**
Reimhult, E.O.: BI+AS+NS-ThA3, 21; BI+AS+NS-ThA8, 22; BI+AS+NS-WeA1, **14**
Rhodes, C.: TF-ThM10, 20
Riehn, R.: BM+MN+MS+TF+BI-ThA8, **23**
Rittschof, D.: BI-TuA9, 8
Roberts, C.J.: BI-ThP20, 28; IJ+BI+MN+SE+AS-ThM10, 18
Romero-Ortega, M.: BM+MN+MS+TF+BI-ThA3, 23
Rosenhahn, A.: BI-TuA8, **8**
Rossi, F.: AS2+BI-ThM6, 17; BI+AS+NS-FrM5, 29
Routaboul, L.: BI+AS+NS-FrM6, 29
Rubal, M.: NS+BI-MoM1, 2
Ruggeri, Z.M.: BI-MoA9, 5
Ruthenburg, T.C.: BI+AS+NS-WeA12, **15**

— S —

Saaem, I.: IJ+BI+MN+SE+AS-ThM11, **18**
Sahin, O.: BI+AS+BM+MS-WeM12, 13
Sandstrom, A.M.: BI-TuP11, **11**
Sanz, F.: AS2+BI-ThM11, 17
Scarbrough, D.: IJ+BI+MN+SE+AS-ThM5, 18
Schiess, A.: BM+MN+MS+TF+BI-ThA3, **23**
Schilp, S.: BI-TuA8, 8
Schneider, J.M.: TF-ThM3, 19
Scholl, A.: BI-TuM6, 7
Schreiner, S.M.: BI-TuP15, 12
Schroeter, A.: TF-ThM4, **19**
Schunk, D.: BI+AS+NS-ThA4, 21
Schvartzmann, M.: BI+AS+NS-FrM3, 29
Scoutaris, N.: IJ+BI+MN+SE+AS-ThM10, **18**
Sengupta, D.: BI-MoA6, **4**
Shah, R.K.: BI-TuP4, 10
Shamloo, A.M.: BM+MN+MS+TF+BI-ThA9, **23**
Shavorskiy, A.: BI-TuM4, 6
Shearer, J.C.: TF-ThM5, **19**
Sheehan, P.: NS+BI-MoM5, **2**
Sheetz, M.P.: BI+AS+NS-FrM3, 29
Shivapooja, P.: BI-TuP3, **10**
Shudy, D.F.: BI-TuP15, 12
Shukla, N.: NS+BI-MoM8, **3**
Shumaker-Parry, J.: BM+MN+MS+TF+BI-ThA6, **23**
Simons, S.: BI-TuP7, 10
Sirghi, L.: AS2+BI-ThM6, 17
Sivaraman, B.: BI-MoA10, **5**
Sjövall, P.: BI+AS+NS-WeA4, 14
Smith, D.R.: BI+AS+NS-WeA10, 15
Sobek, J.: BI+AS+NS-ThA3, 21
Staggs, K.: BI-TuP2, **10**
Stellacci, F.: BI+NS-MoM3, **1**
Streng, D.E.: BM+MN+MS+TF+BI-ThA8, 23
Strunz, T.: AS2+BI-ThM4, 16
Stuart, S.J.: BI-TuP12, 11
Subramanian, R.: NS+BI-MoM11, **3**
Sullivan, S.: BI-MoA7, 4
Sun, Y.: IJ+BI+MN+SE+AS-ThM12, **19**
Sushko, M.: AS2+BI-ThM4, 16
Svedhem, S.: BI+AS+NS-WeA4, 14
Syed, S.: BI+AS+NS-ThA6, 21

— T —

Tadidagapa, S.: BI+AS+NS-WeA9, 15
Takahashi, H.: BI+AS+NS-ThA9, 22; BI-ThP13, 26
Takahashi, T.: TF-ThM3, **19**
Tartis, M.: BI-TuP2, 10
Taylor, M.: BI-MoA3, 4
Tendler, S.J.B.: BI-ThP20, 28
Terfort, A.: BI+AS+NS-ThA10, 22
Textor, M.: BI+AS+NS-ThA3, 21; BI+AS+NS-ThA8, 22
Thapa, T.: BI-TuP7, **10**
Theilacker, W.: BI-MoA7, 4
Thomas, H.: BI+AS+NS-ThA10, 22
Tian, J.: IJ+BI+MN+SE+AS-ThM11, 18
Tisone, T.C.: BI+AS+BM+MS-WeM5, **13**
Tizazu, G.: BI-ThP9, 25
Tjong, V.: BI-ThP12, **26**
Tokumasu, F.: AS2+BI-ThM10, 17
Twiss, J.: BI-MoA7, 4

— U —

Urquhart, A.J.: BI-MoA3, 4

— V —

Valsesia, A.: AS2+BI-ThM6, 17; BI+AS+NS-FrM5, 29
Verma, P.: BI-ThP19, **27**
Voeroes, J.: BI+AS+NS-ThA6, 21
Vogtli, M.: AS2+BI-ThM4, 16

— W —

Wagner, H.D.: BI-ThP6, 25
Wahl, K.J.: BI-TuA9, 8
Wallace, D.B.: IJ+BI+MN+SE+AS-ThM1, **17**
Waly, N.: BI-ThP11, 26
Wang, H.: BI-ThP1, **25**
Wang, X.: BI-ThP1, 25
Warman, M.: BI-TuP10, 11
Weidner, T.: BI-TuM9, **7**
Weiss, P.S.: BI+NS-MoM2, 1
Weitz, D.A.: BI-TuP4, 10
Wellinghoff, S.: NS+BI-MoM1, 2
Wen, X.: BI-ThP14, 26
Wiggins, B.C.: NS+BI-MoM10, 3
Willis, D.: BI-MoA7, 4
Wind, S.J.: BI+AS+NS-FrM3, 29
Wingren, C.: BI+AS+BM+MS-WeM1, **13**
Winkler, T.: BI+AS+NS-ThA10, 22
Wode, F.: BI-TuA8, 8
Wright, P.C.: BM+MN+MS+TF+BI-ThA10, 24; BM+MN+MS+TF+BI-ThA4, 23
Wu, D.: BI+AS+NS-FrM6, 29
Wu, P.C.: BI+NS-MoM1, 1

— X —

Xiao, J.: BI+AS+NS-FrM6, 29

— Y —

Yamazaki, K.: BI-TuP8, 11

Yan, M.: BI-ThP1, 25
Yang, J.: BI-MoA3, **4**; IJ+BI+MN+SE+AS-ThM9, 18
Yang, P.: BI-TuP5, **10**
Yarmoff, J.A.: NS+BI-MoM2, 2
Yellen, B.B.: BI-ThP16, 27
Yu, H.: BI-ThP12, 26

— Z —

Zander, N.: BI-MoA7, 4; BI-MoA8, **4**
Zauscher, S.: AS2+BI-ThM5, **16**; BI+AS+NS-WeA10, 15; BI-ThP16, 27; BI-TuP10, 11
Zhang, K.: BI-ThP6, 25
Zhang, P.: BI+NS-MoM2, 1
Zhang, Z.: BI-MoA7, 4
Zhang, Z.Z.: BI+AS+NS-FrM6, **29**
Zharnikov, M.: BI+AS+NS-ThA10, **22**
Zheng, W.: BI-TuM10, **7**
Zhou, D.: AS2+BI-ThM4, 16
Ziskind, D.: BI-ThP6, 25