Biomaterial Interfaces
Room: 202 - Session BI+SS+NC-MoA

Honorary Session for Bengt Kasemo
Moderator: M. Textor, ETH Zürich, Switzerland

2:00pm **BI+SS+NC-MoA1 Self–Assembly of Organic Molecules on Surfaces Studied by STM: Dynamics, Chirality and Self-Organization**, ***F. Besenbacher***, University of Aarhus, Denmark **INVITED**

Adsorption and organization of organic molecules on solid surfaces is central to self-assembly and bottom-up fabrication within nanoscience and technology. The Scanning Tunneling Microscope allows exploration of atomic-scale phenomena occurring on surfaces: Dynamic processes can be followed by fast-scanning STM, and from data acquired at a range of temperatures; detailed information on kinetic parameters can be extracted. In the talk, a number of studies investigating dynamics and organization of organic molecules on metal surfaces will be described, addressing surface diffusion, chiral recognition chiral switching and also the interaction of molecules with chiral sites on a metal surface[1-5]. Finally, the self-assembly of Nucleic Acid (NA) base molecules on solid surfaces has been investigated. I will discuss the fact that Guanine molecules form the so-called G-quartet structure on Au(111) that is stabilized by cooperative hydrogen bonds[6]. Interestingly, cytosine molecules only form disordered structures by quenching the sample to low temperatures, which can be described as the formation of a 2D organic glass on Au(111)[7]. Molecular recognition between complementary nucleic acid (NA) bases is vital for the replication and transcription of genetic information, both in the modern cell as well as under prebiotic conditions, when a dedicated molecular machinery of evolved living organisms had not yet been developed. By means of variable-temperature Scanning Tunneling Microscopy (VT-STM) we show that on a flat metal surface, formation of complementary NA bases pairs is favoured. The C+G mixture resilience to heating is due to the formation of G-C Watson-Crick base pairs. The observation that not the oligonucleotide backbone, but a flat metal surface may be instrumental for specific WC base pairing has interesting implications for the proposed scenarios of the emergence of life.

[1] M. Schunack et al., Phys. Rev. Lett. 88, No. 156102 (2002)
[2] R. Otero et al., Nature Materials 4 779 (2004)
[3] A. Kühnle et al., Nature 415, 891 (2002)
[4] S. Weigelt et al., Nature Materials, 5 11 (2006)
[5] S. Weigelt et al., Angew. Chem. 119, 9387 (2007)
[6] R. Otero et al., Angew. Chem. Int. Ed. 44, 2270-2275 (2005)
[7] R. Otero et al., Science 319 (2008) 312-315 .

2:40pm **BI+SS+NC-MoA3 Interaction of AH Amphipathic Peptide with Lipid Bilayers and Application to the Understanding of Hepatitis C Viral Infection via QCM-D Measurements**, ***C.W. Frank***, *N.J. Cho*, Stanford University, *K.H. Cheong*, Samsung Advanced Institute of Technology, Korea, *J.S. Glenn*, Stanford University **INVITED**

Membrane association of the hepatitis C virus NS5A protein is required for viral replication. This association is dependent on an N-terminal amphipathic helix (AH) within NS5A and is restricted to a subset of host cell intramolecular membranes. The mechanism underlying this specificity is unknown, but it may suggest a novel strategy for developing specific antiviral therapy. Here we probe the mechanistic details of NS5A amphipathic helix-mediated binding to both cellular-derived and model membranes using biochemical membrane flotation and quartz crystal microbalance with dissipation. In both assays, we observed AH-mediated binding to model lipid bilayers. When cellular-derived membranes were coated on the quartz nano-sensor, however, significantly more binding was detected. Biochemical flotation assays performed with trypsin-treated cellular-derived membranes exhibited reduced amphipathic helix-mediated membrane binding, while membrane binding of control Cytochrome b5 remained unaffected. Similarly, trypsin treatment of the nano-sensor coated with cellular membranes eliminated amphipathic helix binding to the cellular membranes while that of a control lipid-binding protein remained intact. These results, therefore, suggest the effect of a protein in mediating and stabilizing the binding of NS5A's amphipathic helix to its target membrane. These results also demonstrate the successful development of a new nano-sensor technology ideal for both studying the interaction between a protein and its target membrane, and for developing inhibitors of that interaction.

3:20pm **BI+SS+NC-MoA5 Tethered Biomolecular Lipid Membranes - a Membrane Mimetic Sensor Platform**, ***W. Knoll***, *I. Köper, R. Naumann, E.-K. Sinner*, Max-Planck-Institute for Polymer Research, Germany

This contribution summarizes some of our efforts in designing, synthesizing, assembling, and characterizing functional tethered lipid bilayer membranes (tBLMs) as a novel platform for biophysical studies of and with artificial membranes or for sensor development, employing, e.g., membrane integral receptor proteins. Chemical coupling schemes based on thiol groups for Au substrates or silanes used in the case of oxide surfaces allow for the covalent and, hence, chemically and mechanically robust attachment of anchor lipids to the solid support, stabilizing the proximallayer of a tethered membrane on the transducer surface. Surface plasmon optics, the quartz crystal microbalance, fluorescence- and IR spectroscopies, and electrochemical techniques are used to characterize these complex supramolecular interfacial architectures with respect to their assembly, their structure and function. We demonstrate, in particular, that these bilayers show the fluid character of a liquid-crystalline membrane with a specific electrical resistance of better than 10 MΩcm2. Then a totally novel approach for the functional incorporation of membrane proteins, i.e., by their cell-free expression and in vitro reconstitution in the presence of tBLMs is demonstrated. We focus on the yeast expression system for the synthesis of the olfactory receptor species OR5 from Rattus norvegicus. By the combination of the corresponding coding DNA with the protein synthesis machinery of a cell-extract (in vitro transcription and translation) we observe spontaneous and vectorial insertion of an interesting example for a membrane protein into a tethered bimolecular lipid membrane: the OR5 receptor as a family member of the G-protein coupled receptors.

4:00pm **BI+SS+NC-MoA7 Tethered Biomolecular Lipid Membranes - a Membrane Mimetic Sensor Pattern II**, ***E.-K. Sinner***, Max-Planck-Institute for Polymer Research, Germany

4:20pm **BI+SS+NC-MoA8 2D Self-Assembly of Annexin-A5 on Lipid Surfaces: Biological Function, Mechanism of Assembly and Biotechnological Applications**, ***A.R. Brisson***, *N. Arraud, R. Bérat, A. Bouter, B. Garnier, C. Gounou, J. Lai-Kee-Him, S. Tan*, CNRS-University of Bordeaux, France **INVITED**

The self-assembly of proteins in 2D arrays at membrane surfaces is a generic strategy used by the cell for the construction of functional supramolecular edifices, e.g. bacterial S-layers, inter-membrane cadherin junctions, etc.. Annexin-A5 (Anx5) is the prototype member of the annexins, a superfamily proteins which share the properties of binding to negatively charged phospholipids in the presence of Ca2+ ions and forming various types of 2D ordered arrays at membrane surfaces. A detailed model of the structure and mechanism of formation of Anx5 2D arrays has been elaborated from EM, AFM and physico-chemical studies on various types of model membranes – liposomes in solution, lipid monolayers at the air-water interface, supported lipid bilayers.[1-4] The long-debated question of the functional role of Anx5 and annexins starts to be elucidated. The unique properties of binding and 2D self-assembly of Anx5 were exploited to develop various types of molecular tools for nanobiotechnological applications in proteomics, diagnosis or drug delivery. Chimerical proteins made of Anx5 fused to an antibody-binding moiety or linked to cell-adhesion peptides allow the construction of 2D platforms for anchoring antibodies, proteins or cells in a controlled orientation and density.[5] Gold particles functionalized with oriented Anx5 or Anx5-fusion proteins are used for labelling membrane fragments exposing phosphatidylserine molecules, such as apoptotic membranes or plasmatic microparticles, opening novel strategies for the separation and the analysis of circulating cell membrane fragments.

[1]F. Oling, W. Bergsma-Schutter and A. Brisson J. Struct. Biol. 2000, 133, 55-63.
[2]Reviakine, I., Bergsma-Schutter, W. and Brisson, A. J. Struct. Biol. 1998, 121, 356-61.
[3]Richter, R.P.; Lai-Kee-Him, J.; Tessier, C.; Brisson, A. R. Biophys. J.2005, 89, 3372-3385.
[4]Richter, R.P.; Bérat, R; Brisson, A. R. Langmuir 2006, 22, 3497-3505.
[6]Bérat, R.; Rémy-Zolghadry, M.; Gounou, C.; Manigand, C.; Tan, S.; Saltó, C.; Arenas, E.; Bordenave, L; Brisson, A. R. Biointerphases, 2007, 2, 165-172.

5:00pm **BI+SS+NC-MoA10 From Surface Science to Biointerfaces to Nanoscience**, ***B. Kasemo***, Chalmers University of Technology, Sweden **INVITED**

The development of surface science can, depending on ones background and focus, be regarded as a bottom up outgrowth of, e.g., solid state physics towards surfaces (structure, electron structure,..) or molecular physics towards interfaces (collision dynamics, adsorption,…), or one can alternatively see it as the result of a top down process, where technologically important areas, such as semiconductor technology, materials science, catalysis and biointerfaces [1], stimulated development of

more knowledge about and better tools to study interface properties and processes. The strength of surface science originates to a large extent from the strong feed back loop between the top down and bottom up processes, connecting a manifold of interesting fundamental questions with a large diversity of applications. Historically the focus of surface science has moved from simple model systems of small molecules on metal surfaces in UHV, to more complex systems in UHV or at higher gas pressures (e.g. in catalysis), to the liquid phase (e.g. electrochemistry), and further to very complex systems (biointerfaces, tribology,…), involving also more complex materials like oxides and polymers. The evolution sketched above is here exemplified by a personal and subjective choice of examples, like surface scattering and charge transfer processes, catalysis, and biomimetic membranes. The "newest" addition on the arena is nanoscience and nanotechnology, which has connected to almost all fields of traditional surface science. Although one can claim in catalysis, and several other fields, that there has always been a "nano-" element, the control of the latter through fabrication and characterization, is what has changed dramatically over the past decade or so. Specific examples chosen here to illustrate this latter development is taken from nanotechnology for sustainable energy [2], namely (i) so called LSPR applications for solar cells and sensing, (ii) metal hydrides, and (iii) exhaust cleaning catalysis and (iv) fuel cells.

[1]Kasemo, B., Biological Surface Science. Surface Science, Vol. 500 (2002) 656.
[2]Zaech M., Haegglund C., Chakarov D., Kasemo B., Current Opinion in Solid State and Materials Science Vol. 10 (2006) 132.

Biomaterial Interfaces
Room: 202 - Session BI-TuM

Protein and Cell Interactions at Interfaces
Moderator: M.R. Alexander, The University of Nottingham, UK

8:00am **BI-TuM1 Multiscale Analysis of Biological Adhesion**, ***D. Leckband***, University of Illinois, *F. Li*, Pololu Corp. **INVITED**
In biological systems, the number of protein bonds mediating cell contacts varies from a few for tethering leukocytes to vessel walls to more than 105 in mature cell-matrix contacts. The characterization of the response of single bonds to a dynamic force provides insights into the physics of noncovalent bond rupture, but the more biologically relevant situation involves the rupture of multiple bonds between extended surfaces. A fundamental question concerns how adhesion between parallel surfaces bridged by multiple, parallel bonds scales with the physical chemical parameters of the protein-ligand bonds. Here I describe theoretical and experimental investigations of the forced separation of two adhesive surfaces linked via a large number of parallel noncovalent protein-ligand bonds. Specifically, we consider how the adhesive force scales with bond parameters (kinetics and affinities) as a function of dynamic loading. These results show that the separation rate relative to the intrinsic relaxation time of the bonds defines three loading regimes and the general dependence of the adhesion on kinetic or thermodynamic parameters of the bonds. In the "equilibrium regime", the rupture force asymptotically approaches the "equilibrium rupture force", which increases linearly with the equilibrium bond energy. In the near-equilibrium regime, the rupture force increases with the separation rate and increasingly correlates with the bond rupture barrier, or the logarithm of the dissociation rate. Far from equilibrium where rebinding is irrelevant, the rupture force varies linearly with the rupture barrier, and hence with the bond rupture barrier. Therefore, the adhesive strength of biological interfaces involving multiple, parallel bonds depends on the loading rate, and the loading conditions in turn determine which molecular parameters scale the strength of the junction.

8:40am **BI-TuM3 Molecular Dynamics Simulation of the Adsorption Behavior of Peptides with Secondary Structure to Functionalized Surfaces**, ***G. Collier***, *S.J. Stuart*, Clemson University, *B.R. Brooks*, National Institutes of Health, *R.A. Latour*, Clemson University
While it is well understood that protein-surface interactions are of fundamental importance for understanding cell-surface interactions, very little is understood at this time regarding the molecular level events that control protein adsorption behavior. Molecular dynamics simulations methods have enormous potential for development as a tool to help understand and predict protein adsorption behavior. We are conducting molecular dynamics simulations to simulate the adsorption behavior of peptides with secondary structure to functionalized alkanethiol self-assembled monolayer (SAM) surfaces. Two types of structured peptides are being studied: (1) an alpha-helix forming peptide with a primary sequence of Ac-L-K-K-L-L-K-L-L-K-K-L-L-K-L-NH2 (LKalpha14), where L is leucine (nonpolar amino acid), K is lysine (positively charged amino acid), and Ac represents an acetylated end-group, and (2) a beta-sheet forming peptide, Ac–L-K-L-K-L-K-L-K-L–NH2 (LKbeta9). Two types of SAM surfaces are represented, (1) a CH3-SAM (hydrophobic surface) and (2) a COOH-SAM surface (negatively charged surface). Simulations are performed with the CHARMM force field and simulation package using explicitly represented solvent (150 mM Na+/Cl- in TIP3P water) with periodic boundary conditions. An advanced sampling method, known as replica-exchange molecular dynamics (REMD), is being applied in our simulations to generate Boltzmann-weight ensembles of states for each peptide-SAM system, with the resulting ensembles providing equilibrated structures of peptide behavior, both in bulk solution, and when adsorbed to each type of SAM surface. The resulting ensembles are then analyzed to provide a theoretical understanding of how the surface influences the secondary structure of both the LKalpha14 peptide and a pair of LKbeta9 peptides. In addition, assessment is also being made to quantitatively assess how each SAM surface and the peptide-surface interactions influence the water structure at the interphase region of the system relative to bulk water conditions. Simulation results are being compared with NMR, SFG, ToF-SIMS, and SPR experimental studies that are being conducted in a collaborative effort with Profs. Castner, Gamble, Stayton, and Drobny at the University of Washington.

9:00am **BI-TuM4 Heat Shock Protein Expression and Cell Membrane Study of Printed Chinese Hamster Ovary Cells**, ***X. Cui***, *T. Boland*, Clemson University
Cell printing as a new cell seeding technology in tissue engineering has been attracting more and more attentions. The advantages of high throughput, automatically controlled, low cost of cell printing has a promising future for tissue engineering. Inkjet printing technology is currently widely used for cell printing. However, the heating and stress to the cells during the printing process may cause cell apoptosis or other physical or genetic changes. We present here a comprehensively study of changes in heat shock protein expression and cell membrane morphogenesis in Chinese Hamster ovary cells printed with thermal inkjet printers. We found the optimal cell concentration for cell printing using the modified HP Deskjet 500 inkjet printer using the HP 51626A ink cartridge was one million cells per ml. The heat shock protein expression of the printed cells has minor difference between the untreated cells and lower than manually heated cells. The cell membrane of printed cells developed pores which allow small molecules such as propidium iodide and dextran molecules (up to 70kD) to pass. We conclude here that cell printing technology can be used for precise cell seeding in tissue engineering fabrication with minor effect and damages to the printed mammalian cells. The printing process caused temporary pores with various sizes to appear in the membranes. This may have promising applications for small molecules transferred into cells for protein expression and drug delivery purpose.

9:20am **BI-TuM5 Bioactivity of Adsorbed Protein Layers Correlated with Adsorbed Orientation and Conformation**, ***K.P. Fears***, *R.A. Latour*, Clemson University
It has been well established that protein interactions at biomaterial surfaces are of critical importance because it is the adsorbed protein layer that dictates how the body responds to an implanted material. In general, cells do not have receptors for synthetic materials thus lack the ability to directly respond to non-biological surfaces. Therefore, the critical factors that influence the subsequent cellular responses are the protein comprised in the adsorbed protein layer, their structures, and their bioactivities. Due to the complexity of protein structure and the inherent difficulty of studying surfaces, elucidating the detailed molecular mechanisms involved in protein adsorption is a daunting challenge. We have developed experimental methods to quantitatively assess the secondary structure of adsorbed protein layers using circular dichroism along with the bioactivity of the adsorbed protein layer using spectrophotometric assays. The following model proteins were investigated on alkanethiol ($HS-(CH_2)_{11}-R$, R = OH, CH_3, NH_2, COOH) modified gold surfaces: hen egg white lysozyme (14.4 kDa, pI ≈ 11, PDB# 1LYZ), xylanase from thermomyces lanuginosus (21.3 kDa, pI ≈ 3.9, PDB# 1YNA), and glucose oxidase from apergillus niger (63.3 kDa, pI ≈ 4.2, PDB# 1CF3). Since CD only provides information about the secondary structure of proteins, amino acid specific chemical modification was used to modify solvent accessible tryptophan residues to provide information about the tertiary structure of the adsorbed proteins and their adsorbed orientations. The results from these studies provide molecular-level insights regarding how surface chemistry influences the adsorbed structure of proteins and how this influences their bioactivity.

9:40am **BI-TuM6 Development of a Novel Biodegradable and Cyto-Compatible Polyurethane for Use as a Bioink in Ink-Jet Printing**, ***C. Zhang***, *N. Brown*, *T. Boland*, Clemson University
Biodegradable polyurethanes (PUs) were synthesized from methylene di-p-phenyldiisocyanate (MDI), polycaprolactone diol (PCL-diol) and N, N-bis (2-hydorxyethyl)-2-aminoethane-sulfonic acid (BES), serving as a hard segment, soft segment and chain extender respectively. We evaluated the effects of this chain extender on the polyurethanes' degradation rate, mechanical properties, hydrophilicity, antithrombogenecity, and ability to support fibroblast cell attachment and growth. The properties were evaluated by comparing these polymers with those having a 2,2-(methylimino)diethanol (MIDE) chain extender. Mechanical testing demonstrated that the PUs containing BES have tensile strengths of about 17 MPa and elongations up to 400%, higher strength and elongation than PUs containing MIDE. In vitro degradation assays showed the presence of sulfonic acid group decrease the degradation rate of the PU containing BES than that of the PU containing MIDE. Cytocompatibility studies showed that all the PUs are nontoxic, and support cell attachment and proliferation. In vitro platelet adhesion assay showed lower platelet attachment on PU containing BES than that on either PU containing MIDE. Additionally, due to the existence of sulfonic acid groups, the BES extended PU became water-soluble in basic condition and insoluble in acidic condition, a phenomenon that is reversible at pH value of 8.7, making this a pH sensitive

polymer attractive for bioprinting applications. By adding acetic acid into an inkjet cartridge and printing it onto basic PU solution, precision fabricated scaffolds were obtained. We will show that these PU scaffolds have preprogrammed pores with fixed sizes of approximately 20 microns. After 5 days cell culture, fibroblasts are seen to attach and proliferate on the porous printed scaffolds, and a number of the cells penetrated into the pores. These results suggest that these PUs are promising candidates as synthetic inks used for customizable fabrication of tissue engineering scaffolds.

10:40am **BI-TuM9 Surface Immobilization and Characterization of Proteins**, *F. Cheng, P.-C. Nguyen, L. Baugh, P.S. Stayton, L.J. Gamble,* ***D.G. Castner***, University of Washington

Immobilized proteins mediate the interactions between a material and its biological environment. We have used XPS, ToF-SIMS, NEXAFS and SPR to investigate protein immobilization onto surfaces containing nitrilotriacetic acid (NTA), N-hydroxysuccinimide (NHS) and maleimide headgroups. NHS surfaces were prepared by self-assembly of NHS ester oligo(ethylene glycol) thiols (NHS-OEG) onto gold. Protein immobilization onto NHS surfaces occurs primarily through the amine groups on the side chains of lysine residues present on the protein surface, resulting in the proteins being immobilized in a random orientation. Mixed monolayers containing NTA headgroups and OEG chains were self-assembled onto a gold surface. The surface concentration of NTA headgroups was 0.9-1.3 molecule/nm^2 in the mixed NTA/OEG monolayers, compared to 1.9 molecule/nm^2 in pure NTA monolayers. The NTA headgroups were slightly reoriented toward an upright position after OEG incorporation. Histagged, proteins were specifically and reversibly immobilized onto Ni(II)-treated mixed NTA monolayers in well-defined orientations. For a humanized anti-lysozyme Fv fragment the amount of reversible, site-specific adsorption varied from 108 - 205 ng/cm^2 with dissociation rates (k_{off}) between $1x10^{-4}$ and $2x10^{-5}$ s^{-1}, both depending on the NTA surface concentration and orientation. The monolayers without Ni(II) treatment exhibited low nonspecific adsorption. ToF-SIMS was used to compare the controlled orientation of histagged proteins on NTA surfaces with the random orientation of proteins on NHS surfaces. Previously studies have characterized the composition and structure of maleimide-ethylene glycol disulfide (MEG) monolayers on gold for the immobilization of single-stranded DNA oligomers (Lee, et al., Analytical Chemistry 79 (2007) 4390.). These same MEG surfaces were used to covalently immobilize cysteine mutants of the Protein G B1 domain. Two mutants were prepared with cysteines located at opposite ends of the Protein G B1 domain. XPS and SPR were used to quantify the amount of each cysteine mutant onto both bare gold and MEG covered gold surfaces. The ToF-SIMS intensity ratios of amino acid fragments with asymmetric distributions in the Protein G B1 domain (ala, asn, gly, leu, met and tyr) were used to show the two immobilized cysteine mutants had opposite orientations. This difference in orientation was observed on both the gold and MEG surfaces.

11:00am **BI-TuM10 Block-oligonucleotide Brushes: Controlled Structure and Recognition Properties**, *A. Opdahl*, University of Wisconsin, *L.J. Whitman,* Naval Research Laboratory, ***D.Y. Petrovykh***, Naval Research Laboratory and University of Maryland, College Park

DNA brushes with unique properties can be prepared using a new immobilization method that is based on the intrinsic affinity of adenine nucleotides for gold (Opdahl et al., PNAS, 104, 9-14, 2007). The general method uses block-oligonucleotides with sequences that follow a d(A_k-T_m-N_n) pattern: a block of k adenine nucleotides [d(A_k)], followed by a block of m thymine nucleotides [d(T_m)], and a short sequence of n (arbitrary) nucleotides [d(N_n)]. These block-oligonucleotides attach to gold via the d(A) blocks and present the rest of the strand for hybridization or for attachment of other molecular recognition ligands. The range of grafting densities produced by this immobilization method can be further extended by co-immobilizing the d(A_k-T_m-N_n) probe DNA with short d(A_l) DNA that act as lateral spacers. We present two applications of this immobilization strategy. In the first, we use d(A) as a means to immobilize d(N_n) probes for hybridization. We find that the strategy results in reproducible and reversible hybridization behavior, offering practical advantages including low cost and resistance to nonspecific adsorption. Moreover, the high degree of control of probe spacing inherent to the method allows us to observe and quantify by both x-ray photoelecton spectroscopy (XPS) and surface plasmon resonance (SPR) the effects that surface density and conformation of DNA probes have on hybridization efficiencies. In the second application, the d(N_n) portion of the strand is replaced with a biotin functionality [d(A_k-T_m-biotin)]. Altering the length or mole fraction of the d(A_l) lateral spacer systematically changes the surface coverage of biotin, allowing control over the amount of streptavidin (SA) that can be linked to the surface. The SA captured by the d(A_k-T_m-biotin) layer is stable and maintains activity towards addition of a subsequent layer of biotin-functionalized molecules. Control experiments also indicate that gold surfaces covered by d(A) oligos exhibit resistance to nonspecific adsorption of SA, both as a molecule and as SA-functionalized microbeads. Since many types of molecules can be functionalized with biotin, compatibility with biotin-SA opens the door to a broad range of applications based on DNA immobilization via d(A) blocks.

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11:20am **BI-TuM11 Surface Initiated Polymer Coatings for the Control of Cell-Surface Interactions**, ***L. Meagher***, *H. Thissen, P. Pasic, R.A. Evans, S. Pereira, G. Johnson, G. McFarland, K. Tsang, T. Gengenbach, K. McLean*, CSIRO Molecular and Health Technologies, Australia

Interest in surface initiated polymerization (SIP) to generate surface coatings for application in biomaterials has increased rapidly in the last few years, particularly those coatings generated using "living" free radical polymerization since they result in coatings with very well defined properties and architectures.[1] Biomaterials applications have largely been in the control of cell attachment, achieved by varying the type of monomer used (e.g. neutral, hydrophilic coatings can be used to reduce protein adsorption and cell attachment). In this study, we present an approach to preparing such coatings which relies on the covalent attachment of a polymer containing controlled free radical initiators onto amine surfaces.[2] This approach allows for maximum flexibility with respect to the substrate (unlike those using self assembled monolayers). Whilst earlier studies have relied on passive coatings, we have included biological signals by building activated functional groups into the coatings as well as drugs and molecules for the specific binding of proteins. These coatings therefore have the potential to interact with biological systems in a much more dynamic manner. XPS and colloid probe AFM were used for coating characterisation. Cell culture studies (24 hours) were carried out with either HeLa, bovine corneal epithelial or L929 fibroblast cells using standard techniques. Protein adsorption was quantified using time resolved fluorescence and proteins labeled with an Eu chelate. Examples presented in this study are the use of iniferters, ATRP initiators and RAFT agents to form SIP coatings with well defined properties. For example, dense polymer brushes which have low or high cell attachment depending on the type and amount of protein adsorption. In addition, the incorporation of cell attachment peptides into coatings via reaction with activated NHS esters will be discussed. Furthermore, the use of click chemistry to conjugate biological signals into coatings formed using our approach will be presented as well as the incorporation of molecules which allow for specific protein binding (e.g. polymerisable biotin) will be discussed in the form of application to ELISAs.

[1] Edmond, S., Osborne, V.L. and Huck, W.T.S. Chemical Society Reviews, 33, 14, 2004.

[2] Meagher, L., Thissen, H., Pasic, P., Evans, R.A., Johnson, G., Polymeric coatings and methods for forming them, WO2008019450-A1, 21 Feb 2008.

11:40am **BI-TuM12 The Effects of Plasma-Polymerized Surface Interactions on R1 Mouse Embryonic Stem Cell Differentiation**, ***E. Hanley***, *J.L. Lauer, G.E. Lyons, J.L. Shohet*, University of Wisconsin-Madison

Plasma polymerized tetraglyme coatings have been studied for their non-fouling and biocompatibility properties due to their inhibition of non-specific protein binding. In this work, we explore how embryonic stem (ES) cell differentiation patterns are affected by surface interactions with various plasma-processed materials. In order to identify a surface as a potential scaffolding material for ES cells in the development of an artificial blood vessel, at least two design criteria must be met. First, the ES cells must adhere to the surface. Second, the surface must facilitate, rather than inhibit, the process of vasculogenesis. ES cells were plated on the following surfaces to compare their effectiveness: 1.) glass cover slips onto which a coating of tetraglyme was deposited by plasma polymerization, 2.) vacuum gas plasma treated polystyrene (Falcon), and 3.) unprocessed control glass coverslips (Corning). In order to characterize the progression of differentiation of the ES cells, each sample was fixed three and seven days after cells had been plated on each surface, and then stained for immunofluorescence analysis. Two genetic markers were used for the antibody staining procedure. PECAM (platelet endothelial cell adhesion molecule: CD31) is an early marker for endothelial cell differentiation. vWF (von Willebrand Factor) is a cytoplasmic protein only expressed in mature endothelial cells. It has been previously reported by using an RT-PCR/southern hybridization blot analysis that ES cells in embryoid bodies first express PECAM near day 5 of differentiation and will first express vWF near day 11 of differentiation. ES cells that were plated on tetraglyme surfaces showed expression of PECAM after 3 days and vWF after 7 days of being plated on the surface. The positive results seen by ES cell derivatives precociously expressing the vWF and PECAM genetic markers on the surfaces suggest a directed differentiation of ES cells into endothelial cells. Furthermore, by using confocal microscopy, it was shown that the endothelial cells that express the vWF genetic marker are those cells closest

to the tetraglyme coating. We conclude that the nature of the surface does influence R1 stem cell differentiation. It has also been shown that higher mole fractions of tetraglyme in the plasma during processing increases the percentage of endothelial cell expression.

Synchrotron-based Spectroscopy and Spectro-Microscopy Topical Conference
Room: 310 - Session SY+SS+BI-TuM

Synchrotron-based Spectroscopy and Spectro-Microscopy
Moderator: M. Grunze, University of Heidelberg, Germany

8:00am **SY+SS+BI-TuM1 X-ray Studies of Hydrogen Bonding in Water; the Liquid Phase and on Surfaces**, ***A.R. Nilsson***, SSRL/Stanford University and Stockholm University, Sweden **INVITED**

Water and its ability to form Hydrogen bonding (H-bonding) is the basis for all life on the planet earth. The understanding of water adsorption, wetting and reactions at solid surfaces is of importance for many different areas of science such as biomaterials, catalysis, electrochemistry, corrosion, environmental science and technologies related to hydrogen as a future energy carrier. There are recent experiments that have raised the question whether we really understand the nature of H-bonding and the structure of liquid water. We have recently devoted a major effort to the development of x-ray spectroscopy measurements of water in the different aggregation forms and adsorbed on surfaces. Using x-ray absorption spectroscopy (XAS), x-ray Raman scattering (XRS), x-ray emission spectroscopy (XES), small angle x-ray scattering (SAXS) and x-ray diffraction together with density functional theory (DFT) calculations we have demonstrated the appearance of specific spectral features that can be related to two different types of water species in the liquid, tetrahedral water and asymmetric H-bond configurations. The latter species dominates the liquid. I will address fundamental questions regarding geometric structure, electronic structure, nature of surface chemical and hydrogen bonding and reactivity of water on surfaces. The connection between studies performed at both UHV and ambient conditions will be emphasized. Several examples of different water adsorption system will be illustrated such as Pt(111), Ru(001), Cu(110), Cu(111), TiO_2, Fe_2O_3 and MgO.

8:40am **SY+SS+BI-TuM3 Soft X-ray Spectroscopy of Liquids and Liquid-Solid Interfaces**, ***C. Heske***, University of Nevada Las Vegas **INVITED**

With the advent of high-brightness synchrotron radiation in the soft x-ray regime it has become possible to investigate vacuum-incompatible sample systems such as liquids using suitably designed in-situ cells. In such cells, thin membranes (e.g., made of SiC, SiN_x, or polyimide) separate non-vacuum sample environments from the ultra-high vacuum necessary for soft x-ray synchrotron beamlines. If the membranes are thin (e.g., on the order of 100 nanometer to 1 micrometer), it is possible to transmit soft x-rays with sufficient intensity for spectroscopic experiments. Two experimental techniques are of particular interest, namely x-ray absorption spectroscopy (XAS) to study unoccupied electronic states and x-ray emission spectroscopy (XES) to investigate the occupied electronic levels. The combination of the two approaches, i.e., the collection of XES spectra at variable resonant excitation (resonant inelastic soft x-ray scattering - RIXS), gives unprecedented insight into the electronic structure of hitherto inaccessible samples, such as liquids and liquid-solid interfaces. In this presentation, the experimental requirements for such studies will be discussed and it will be demonstrated how XAS, XES, and RIXS can give unique insights into the electronic, chemical, and dynamic properties of liquids (in particular water) and liquid-solid interfaces.

9:20am **SY+SS+BI-TuM5 High-Resolution X-Ray Photoelectron Spectroscopy as a Versatile Tool for the Characterization of Monomolecular Self-Assembled Films**, ***M. Zharnikov***, Universität Heidelberg, Germany **INVITED**

Self-assembled monolayers (SAMs) have recently attracted considerable interest in physics, chemistry and biology due to their ability to control wetting, adhesion, lubrication and corrosion on surfaces and interfaces and their capability to become building blocks of future electronic devices. All the above applications rely on deep understanding of properties of these systems and precise knowledge of their structure. We will review recent progress in characterization of SAMs with a chalcogen headgroup on coinage metal and semiconductor substrates by high-resolution X-ray photoelectron spectroscopy (HRXPS). As compared to conventional XPS with a laboratory X-ray source, HRXPS, which usually takes advantage of both ultimate energy resolution and tunable photon energy (synchrotron), is capable to deliver additional information on the objects of interest, including screening phenomena, homogeneity of the bonding configurations, charge transfer upon the headgroup-substrate bond formation, etc. Also, this technique is very useful when dealing with systems exhibiting a large diversity of chemical species such as, e.g., SAMs on GaAs substrates. A variety of examples will be provided. Further, it will be shown that photoemission in SAMs cannot always be described within the standard theoretical framework, which have important implications for both understanding of the XPS/HRXPS spectra of the relevant SAM-derived systems and practical applications.

10:40am **SY+SS+BI-TuM9 Chemical Imaging and Spectroscopy at Sufficiently High Spatial Resolution to Uncover Functions of Nanoscale Phenomena**, ***M. Kiskinova***, Sincrotrone Trieste, Italy **INVITED**

The complementary capabilities of different microscopy approaches in terms of imaging, spectroscopy, spatial and time resolution are strongly requested by the multi-disciplinary research programs at the synchrotron facilities and have motivated continuous investments in development of instrumentation for imaging with spectroscopic analysis. The major part of the lecture will be focused on the potential of modern x-ray photoelectron microscopes in chemical imaging and micro-spot photoelectron spectroscopy.[1] Among the selected research topics, as representative examples are (i) addressing the surface properties of the individual C and oxide nanostructures and supported catalyst micro and nano-particles (ii) mass transport driven self-reorganization processes which can introduce lateral heterogeneity in the composition and reactive properties of surfaces (iii) quantum-size effects on the local chemical reactivity measured for ultrathin films with spatially varying thickness. The final part of the lecture will briefly illustrate the most recent achievements in combining the potential of soft x-ray transmission microscopy with multiple contrast approaches and fluorescence analysis.

Günther, S., Kaulich B., Gregoratti L., Kiskinova, M.: Prog. Surf. Sci. 70, 187, 2002.

11:20am **SY+SS+BI-TuM11 Hard X-ray Photoelectron Spectroscopy up to 15 keV: State-of-the-Art and Recent Results**, ***M. Merkel***, FOCUS GmbH, Germany, *J. Rubio-Zuazo, G.R. Castro,* SpLine Spanish CRG Beamline at the European Synchrotron Radiation Facility, France, *M. Escher*, FOCUS GmbH, Germany

Hard X-ray photoelectron spectroscopy (HAXPES) gains momentum as a new non-destructive nanoanalytical method more and more. On one hand the request for non destructive and bulk sensitive analysis methods is highly visible. On the other hand the availability of a number of suitable high energy synchrotron beam lines allows for the realization of such dedicated instrumentations. A new electron analyzer that fulfils the requirements imposed by the XRD and HAXPES techniques is presented. The analyzer of the cylindrical sector type[1] in use (FOCUS HV CSA) is a very compact and at the same time highly efficient approach for this kind of electron spectroscopy. It is capable to handle kinetic energies up to 15 keV down to a few eV with the same analyzer setup and power supply.[2] The recent implementation of a 2D event counting detector for parallel data acquisition will be described also. By means of this detector the measurement speed is increased to overcome the restrictions imposed by the reduced sample cross sections and analyser transmission at high kinetic energies. Buried layers, as they are common for a number of nanotechnological applications, are invisible with most of the known non destructive analytical methods. To demonstrate the potential of electron spectroscopy at really high kinetic energies we used thin Au layers deposited onto a Cu substrate as a model system for bulk sensitive photoemission. The comparison of the Cu3s and Au5s peaks show the energy dependence of the depth information. It is seen that substrate properties can be probed for kinetic energies $\geq$ 10 keV effectively. By means of such measurements we derived the energy dependence of the effective attenuation length (EAL) for electrons in Au also. For this purpose core level spectra of different energies are taken for different film thicknesses. The extracted EAL of Au shows an energy dependence of $(Ekin)^{0.622}$ what is in good agreement with literature data. This work was supported through the Spanish Ministry of Education and Science (MEC), grants nos. FAP-2001-2166 and MAT1999-0241-C01 and the German Ministry of Education and Research (BMBF) under grant no. FKZ 13N9033.

[1] Risley J.S, Rev. Sci. Instrum. 43 (1971) 95; Sar-El H.Z, Rev. Sci. Instrum. 38 (1967) 1210; Sar-El H.Z, Rev. Sci. Instrum. 41 (1970) 561.

[2] J.R.Rubio-Zuazo, M.Escher, M.Merkel and G.R.Castro, J. of Phys. Conf. Ser. 100 (2008).

11:40am **SY+SS+BI-TuM12 A Comparative Study of Interface Formation for Ca/PDHFV and Ca/PHF by Synchrotron Radiation Photoemission**, *Y.X. Guo, W. Zhao, X.F. Feng, L. Zhang, W.H. Zhang,* ***J.F. Zhu***, University of Science and Technology of China

Interfaces of metal/polyfluorene have attracted much research interests in both technological and scientific point of view. Polyfluorene-based light

emitting devices have been proved to have a high luminescene effciency. However, the occurrence of gap states in most cases will affect the luminescence properties of organic materials. In this paper, the interface formation and energy level alighment for Ca/PDHFV and Ca/PHF have been studied by synchrotron radiation photoemission spectroscopy (SRPES) and X-ray photoelectron spectroscopy (XPS). The results imply that the chemical reaction at the interface of Ca/PDHFV is stronger than that of Ca/PHF. However, no gap states at the Ca/PDHFV interface can be observed, which is different from the observations on Ca/PHF. Both of these two interfaces display low electron injection barrier. Our findings suggest that the gap states can be removed at metal/polyfluorene interface by introducing vinylene units into polyfluorene, which may provide a new way to eliminate the gap states.

Biomaterial Interfaces
Room: 202 - Session BI+NC-TuA

Protein and Cells Interactions on Micro- and Nanofabricated Substrates

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

1:40pm **BI+NC-TuA1 Exploring Single Stem Cell Biology via Microarrayed Artificial Niches**, ***M.P. Lutolf***, Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland, *R. Doyonnas, H.M. Blau*, Stanford University **INVITED**

A complex mixture of extracellular cues delivered by support cells is critical for adult stem cell maintenance and regulation of self-renewal in their microenvironment, termed niche. Despite recent progress in the identification of relevant niche proteins and signaling pathways in mice, to date, hematopoietic stem cells (HSCs) cannot be efficiently cultured in vitro without rapidly differentiating. We are developing novel in vitro culture paradigms that allow fate decisions of individual stem cells to be monitored under well-controlled conditions and in real time. We have engineered microarrayed artificial niches based on a combination of biomolecular hydrogel and microfabrication technologies that allow key biochemical characteristics of adult stem cell niches to be mimicked and the physiological complexity deconstructed into a smaller, experimentally amenable number of distinct signaling interactions. Several thousand single stem cells were tracked by fluorescent time-lapse microscopy in these microarrays over a period of several days. Image analysis allowed individual cell fate changes and growth kinetics of entire populations to be statistically analyzed. Subsequent retrospective single cell RT-PCR and transplantation experiments were performed in order to correlate kinetic behavior with phenotype and function. Screening of ca. 20 putative soluble HSC regulators, including Wnt-3a and TPO, as well as surface-tethered cell-cell adhesion proteins such as N-Cadherin, allowed to identify factors that dictate distinct HSC cell cycle kinetics. Based on patterns in kinetic behavior and single cell gene expression profiles induced by stimulation with a few of these candidates, we distinguished hallmarks of self-renewal from differentiation divisions, and validated these disparate behaviors in vivo by subsequent HSC transplantation into lethally irradiated mice. Therefore, the systematic deconstruction of a stem cell niche may serve as a generalizeable paradigm for defining and reconstructing artificial niches to accelerate the transition of stem cell biology to the clinic.

2:20pm **BI+NC-TuA3 Highly Ordered Protein Patterns Generated from Self-assembly of Mixed Protein Coated Nanoparticles**, ***G. Singh***, *S. Pillai, A. Arpanaei, P. Kingshott*, University of Aarhus, Denmark

The generation of protein patterns with controllable spacing in the nanometer to micrometer range is of great interest particularly for gaining a more fundamental understanding of the molecular mechanisms associated with protein-surface interactions. Information which can provide new insights into how proteins regulate cell shape and function have great interest in many areas including medical implants, tissue engineering, sustained drug delivery devices, biofilms, and biosensors. We present here a new but simple method for generating highly ordered protein patterns that can be applied over large areas (cm^2) based on the self-assembly of mixed nanoparticles of different size, from very low volume fractions. The proof concept experiments initially involve separately coating the particles with a different type of protein. The particles are then mixed at variable concentrations at low total volume fraction. Experiments are performed with fluorescently labelled proteins. For example, FITC-labelled lysozyme is adsorbed to 2µm carboxylated polystyrene particles and rhodamine-labelled BSA is adsorbed to 0.2µm aminated polystyrene particles both in PBS at pH=7.4. In separate experiments either both types of protein-coated particles are mixed in solution, or one protein coated particle is mixed with an uncoated particle. The particle suspensions are drop-cast onto the centre of a rubber ring placed on a hydrophilic Si wafer substrate. A concave shaped layer of solvent is formed allowing for long range assembly of binary colloidal particles firstly through lateral capillary forces, and later by convective flow and entropic forces. The resultant patterns consist of highly ordered hexagonal arrays of large particles as a single layer encapsulated by crystals of the smaller particles, in which single or binary proteins patterns are generated. The coverage is simply controlled by calculating the area of particles needed to fill the rubber ring, and the spacing is controlled by the appropriate ratio of starting particles. The ordered protein patterns are shown using fluorescent microscopy combined with AFM and SEM analysis. The extent of protein adsorption to the particles is determined using XPS and zeta potential measurements. The method is very simple, fast and inexpensive and we demonstrate that the patterning of proteins covers the range for a few nanometers up to a few micrometers depending on the appropriate selection of particles.

2:40pm **BI+NC-TuA4 Laminin Functionalization onto Silicon Single Crystals and Silicon Templated in Molecule Corrals**, ***S.P. Sullivan***, *X. Zhang, M.E. Boggs, H.P. Bui, A.V. Teplyakov, T.P. Beebe, Jr.*, University of Delaware

Biological and chemical surface modifications at the nanoscale have become a large area of research in response to the need for new and improved applications such as biochemical sensors and medical implants. The work describe here investigates the important extracellular matrix protein, Laminin onto Si(111) and templated silicon nanostructures. These substrates are being evaluated as biomaterial bridges for neuron outgrowth. The nanostructures are templated onto the surface of highly oriented pyrolytic graphite using "molecule corrals," which are nanometer-sized (1 – 100 nm diameter) structures etched into the basal plane of graphite. The intial defects from which molecule corrals originate are routinely produced using a low-energy cesium ion beam, followed by thermal oxidation at 650 ˚C. Using a physical vapor deposition method, silicon is then deposited onto the HOPG, leading to the formation of billions of silicon nanostructures. Previous results suggest that these structures will react similar to that of hydrogen-terminated silicon single crystal wafers. A comparison with a new protein attachment scheme, beginning with a self-assembled monolayer of 11-amino-1-undecene, was completed. XPS, TOF-SIMS, and AFM were used to characterize the substrates following each step of the reaction. To avoid deposition of physically adsorbed protein, careful rinsing and sonication procedures were optimized and used. From these results it was determined that the nanostructures react similarly to the hydrogen-terminated Si(111) surface for this covalent attachment scheme, and that protein attachment was successful on the nanostructures. To evaluate the reaction efficiency, an additional study comparing two covalent protein attachment schemes on silicon nanostructures is underway.

3:00pm **BI+NC-TuA5 Characterisation and Patterning of PEG-Supported Lipid Bilayers**, ***S. Kaufmann***, *P. Spycher, K. Kumar*, LSST, ETH Zurich, Switzerland, *G. Papastavrou*, LCSC, University of Geneva, Switzerland, *M. Textor, E. Reimhult*, LSST, ETH Zurich, Switzerland

Supported lipid bilayers (SLB) provide a basis for biotechnological applications as they constitute a simple model of cell membranes. They are of particular interest as components of future generations of biosensors based on transmembrane proteins. Two of the current limitations of supported lipid bilayers in biosensor applications are their sensitivity to air exposure and the limited aqueous space between the sensor substrate and the membrane available for large membrane proteins. Supported membranes resting on a hydrophilic polymer spacer decouple the membrane from the surface and provide increased aqueous space, but are generally more complicated to assemble than supported lipid membranes resting on an inorganic support. Recently it has been shown that poly(ethylene glycol) (PEG) can be incorporated into the membrane of liposomes through lipid molecules end-functionalized with a PEG chain and spontaneously fused to supported PEG-lipid bilayers (PEG-SLB) on glass. These membranes have been shown to possess a remarkable stability in air and would based on the length of the PEG-chains provide enough space between the SLB and the substrate to allow incorporation of functional transmembrane proteins. However, the structure of the PEG-SLB has not been characterized and important questions like whether the PEG brush is present on both sides of the membrane, its thickness, density and the kinetics of formation of PEG-bilayers have not been addressed. We present a comparison of the kinetics of PEG-SLB formation for different PEG molecular weights and densities as well as structural information. Furthermore, patterning of PEG-SLB using microspotting in glycerol-containing buffer has been done and compared to that of phosphocholine (PC) SLBs. QCM-D and FRAP measurement indicate decreased efficiency of PEG-SLB formation with increased PEG-density. This is most apparent in the initial adsorption of PEG-liposomes suggesting that POPC lipids still drive SLB formation through a mechanism similar to pure POPC SLBs and that a higher screening of the POPC lipids by PEG chains decreases the surface interaction. Force spectroscopy measurements demonstrate the presence of PEG on both sides of the SLB. SLB formation could be facilitated in glycerol-containing buffer and spotting of PC-SLBs and PEG-SLBs obtained by hydration, but with low geometrical definition. Spotting and hydration of PEG-SLBs demonstrated a weaker adhesion of PEG-SLBs than PC-SLBs.

4:00pm **BI+NC-TuA8 Nano-Rough Surfaces Produced by Glancing Angle Deposition (GLAD) for Protein Adsorption Measurements and Cellular Assays**, *A. Dolatshahi-Pirouz,* Univ. of Aarhus, Denmark, *C.P. Pennisi,* Aalborg University, Denmark, *S. Skeldal,* ***M. Foss****, J. Chevallier, P. Kingshott,* Univ. of Aarhus, Denmark, *V. Zachar,* Aalborg University, Denmark, *K. Yoshida,* Indiana University and Purdue University, *F. Besenbacher*, Univ. of Aarhus, Denmark

Currently, there is a strong focus on the fabrication of nanostructured artificial surfaces in order to tailor the biological response of artificial materials. The nanostructures are mainly used for more fundamental protein and cell studies, but in some cases also for applications like implants and cell/tissue engineering. Here a simple method to generate nano-rough platinum surfaces with varying morphological characteristics and a well-controlled surface roughness has been employed. The surfaces were fabricated by glancing angle deposition (GLAD) with varying angles and deposition times. Afterwards the biological response of the characterized nanorough samples were examined by protein adsorption and cell adhesion/proliferation assays in order to evaluate their potential as biomaterials surfaces. The effect of the deposition angle, θ, and deposition time, t, on the morphological characteristics of the thin films was investigated by utilizing Atomic force microscopy (AFM) and analyzing the images in order to determine the surface roughness and the size of the nano-rough surface features. The chemical composition of the platinum coatings were examined by X-ray Photoelectron Spectroscopy (XPS). From the AFM images it is observed, that the surface nano-features residing on the substrates can be changed by varying the deposition angle: as the deposition angle approaches grazing incidence sharp columnar protrusions are grown, while more smoothly shaped surface features appear for the thin films fabricated at higher deposition angles. The surface root-mean-square roughness, w_{rms}, increased from 1.49 nm to 15.2 nm as grazing incidence was approached. The surface roughness was additionally enhanced from w_{rms} = 6.6 nm to 26.3 nm for films grown at θ = 5° by increasing the deposition time. It is found that the blood fluid protein, fibrinogen, is influenced by the nano-rough substrates as compared to a flat control surface. Furthermore, the proliferation of primary human fibroblasts is almost completely inhibited on the nano-rough substrates. A maximum difference of almost 200% is observed between the tallest columnar surface features (44 ± 5 cells/mm2) and the flat platinum reference (125 ± 6 cells/mm2). These results show that GLAD is a versatile technique for fabrication of varying nano-rough model surface morphologies capable of influencing both the protein and cell behavior on the surface.

4:20pm **BI+NC-TuA9 HaloTag™ Protein Arrays: An Integrated Biomolecular Interaction Analysis Platform**, ***N. Nath****, R. Hurst, B. Hook, K. Zhao, D. Storts, B. Bulleit*, Promega Corporation

Protein arrays are emerging tools geared toward proteome wide detection of protein-protein, protein-drug, protein-DNA or protein-antibodies interactions. Wide application of protein array technology however faces significant challenge due to lack of high-throughput method for protein expression and purification. Here we present a new integrated approach for creating protein arrays that combines in-vitro protein expression system with HaloTag™ capture technology. The method allows for rapid and covalent capture of HaloTag™ fusion proteins in an oriented fashion directly from complex protein matrices without any prior purification. Multiple fusion proteins can be rapidly synthesized (90min) and immobilized in parallel for high throughput studies. We also demonstrate that arrayed fusion proteins are functionally active and can be used for protein-protein and protein-nucleic acid interaction studies. Furthermore, we show that by using a HaloTag-Protein G fusion we can fabricate antibody arrays directly from ascites fluid without any prior purification of antibodies. Unlike current antibody array platforms, antibodies on our platform are oriented on the surface for maximum biological activity. HaloTag™ protein arrays thus provide a single platform for multiple-biomolecular interaction studies.

4:40pm **BI+NC-TuA10 Use of Aligned Polymer Microfibres for Peripheral Nerve Repair**, *C. Murray-Dunning, R. McKean, A.J. Ryan, S.L. McArthur,* ***J.W. Haycock***, Sheffield University, UK

Nerve guidance conduits (NGC) have considerable potential for repairing peripheral nerve gap injuries caused by trauma, with basic entubulation designs encouraging limited reinnervation of nerve fibres. Following transection injury, Schwann cells are essential for repair as they proliferate rapidly, clear debris and secrete growth factors. We have designed a closed loop bioreactor enabling us to seed Schwann cells into experimental NGCs comprised of uniaxially aligned poly-L-lactide microfibres. Cells were introduced in fibres (5-10µm diameter) varying in length from 10-80mm, contained within 1.2mm diameter silicone tubes and grown under static and flow conditions (0-5.0ml/min) for 24 - 96 hours. MTT and confocal live/dead analysis data showed that cell viability was considerably improved when given an initial 4 hour adhesion time followed by a 0.5ml/min flow rate. To optimise Schwann growth within aligned fibre scaffolds, we then investigated seeding cells onto aligned fibres which were surface modified by acrylic acid plasma deposition. Schwann cells were stained with live/dead and phalloidin-FITC fluorophores and analysed by confocal microscopy in 3D. Microfibre scaffolds revealed a high degree of uniaxial cell alignment and a 50% increase in cellular viability on acid surface treated fibres, verse uncoated PLLA fibres. In conclusion, the following NGC approach is readily adaptable for autologous and stem cell delivery methods for the pre-clinical investigation of 3D tissue models for peripheral nerve repair.

5:00pm **BI+NC-TuA11 The Synthesis of Smooth PZT Thin Films and the Effects of Self-Assembled Monolayers and Ferroelectric Polarization on Surface Properties**, ***R.E. Ducker****, A. Garcia, B.B. Yellen, S. Zauscher*, Duke University

Lead zirconium titanate (PZT) thin films have attracted a great deal of interest in recent years due to their piezoelectric and ferroelectric properties. Recent applications for these films are in microelectromechanical systems (MEMS) and ferroelectric non-volatile random access memories. Here we present the formation of self-assembled monolayers (SAMs) on thin polarizable PZT films for potential biological applications such as biosensors. PZT Pb(Zr0.5Ti0.5)O3 thin films were prepared by a sol-gel deposition on platinum coated silicon substrates. The surface properties of the platinum substrate were modified using SAMs and the effects on the final crystal structure was investigated. These films were characterized by X-ray diffraction, scanning electron microscopy, X-ray photoelectron spectroscopy (XPS). We also present the formation of SAMs of alkylphosphonic acids and alklysilanes on the surface of these PZT films. Monolayers on metal oxide surfaces are an important way of changing the surface chemistry of functional materials. The formation of SAMs on PZT is not well understood compared to other metal oxide systems. These monolayers were formed with varying surface chemistries. These were characterized by contact angle goniometry, XPS and atomic force microscopy (AFM). Thirdly we present the ferroelectric polarization of the thin PZT films. The ferroelectric domains of PZT can be polarized using a conductive AFM tip. A DC bias voltage was applied between the tip and the bottom Pt electrode on the substrate to achieve features ranging from 400nm to several microns. The characterization of the polarization was performed using scanning Kelvin probe microscopy, electric force microscopy and scanning polarization force microscopy. These techniques confirmed the presence of an out-of-plane polarization component due to the reorientation of the ferroelectric domains in the PZT. The ability to change the polarization of PZT back and forth by applying opposite polarities to the AFM tip is also demonstrated. The effect of the polarization on the monolayer is also discussed.

5:20pm **BI+NC-TuA12 Patterned Protein Gradients of Extracellular Matrix Protein Affect Cell Attachment and Axonal Outgrowth**, *W.M. Theilacker, A.L. Styer,* University of Delaware, *D.E. Willis, J.L. Twiss,* Alfred I. DuPont Hospital for Children, *M.E. Boggs, S.P. Sullivan,* University of Delaware, *Z. Zhang,* Spansion, Inc., ***T.P. Beebe, Jr.***, University of Delaware

We have developed a method to control the local surface density of peptides and proteins that are covalently attached to various test substrates, for cell culture assays ranging from cell attachment propensity, to cell attachment density, to cellular behavior and signaling, to cell-cell interactions, for a variety of cell types and for a variety of proteins and peptides. This degree of control has recently been extended to step and continuous gradients in local protein and peptide concentrations from the micron to the centimeter length scale. Our use of these test substrates has focused mainly on neuronal cell types, for the development of new biomaterial bridging applications in brain and spinal cord injury patients, and on cell-cell interactions between osteocytes and neurons, for an understanding of "bone pain" in cancer patients. This presentation will focus on the production of these test substrates, their characterization by a variety of surface analytical and optical microscopy techniques, including XPS, TOF-SIMS, AFM, and epi-fluorescence microscopy with immunostaining, and the results of cell culture studies using these test substrates.

Biological, Organic, and Soft Materials Focus Topic
Room: 201 - Session BO+PS+AS+BI+SS-TuA

Plasma-deposited Polymer and Organic Surfaces in Biological Applications

Moderator: E.R. Fisher, Colorado State University

1:40pm **BO+PS+AS+BI+SS-TuA1 High Throughput Surface Chemical Analysis of Polymer Microarrays: Wettability, Protein Adsorption and Cell Response Correlations**, *M. Taylor, A.J. Urquhart,* The University of Nottingham, UK, *Y. Mei, D.G. Anderson, R. Langer,* MIT, *M.C. Davies, **M.R. Alexander***, The University of Nottingham, UK **INVITED**

In the search for new and improved biomaterials, combinatorial material discovery approaches are increasingly being explored. A significant development in the production of polymer libraries by parallel synthesis was the move from preparation of macroscopic samples,[1] to on-slide polymerisation as microarrays in nano-litre volumes.[2] Such microarray material libraries may readily be interrogated by automated surface analysis equipment. Recently, high throughput surface analysis of a library of 576 different acrylate copolymers in triplicate on one slide using water contact angle (WCA), XPS and ToF SIMS highlighted the difference in the bulk and surface composition of the polymer spots, and consequently the need for surface analysis data when determining structure-property relationships.[3] The complexity of SIMS data, multiplied by the number of different samples necessitates the use of multivariate analytical approaches. Using partial least squares (PLS) analysis, relationships between SIMS fragments and WCA have led to identification of moieties controlling wettability across the wide range of copolymers synthesised on one microarray.[4] Comparison of human embryonic stem cell number on the spots with SIMS spectra have identified further SIMS fragments that correlate with high or low cell-polymer affinity. Protein adsorption measurements have been undertaken in an attempt to rationalise the cell adhesion data.[5] The correlations identified, and the information on the relationship between the surface structure and cell response or wettability will be discussed in this exploration of the high throughput approach.

[1] Brocchini S et al. Structure-property correlations in a combinatorial library of degradable biomaterials. Journal of Biomedical Materials Research 1998 42 66.

[2] Anderson DG, et al. Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells. Nature Biotechnology 2004 22 863.

[3] Urquhart AJ, et al. High throughput surface characterisation of a combinatorial material library. Adv Mats 2007 19 2486.

[4] Urquhart AJ et al. TOF-SIMS analysis of a 576 micropatterned copolymer array to reveal surface moieties that control wettability. Anal Chem 2008 80 135.

[5] Taylor M et al. A Methodology for Investigating Protein Adhesion and Adsorption to Microarrayed Combinatorial Polymers. Rapid Macromol Comm 2008 (in press).

2:20pm **BO+PS+AS+BI+SS-TuA3 Plasma Medicine**, ***A. Fridman***, Drexel University **INVITED**

Novel engineering and science approaches sustaining human health, such as for example radiation biology and laser medicine, represent a significant segment of technological developments around the world. Recent breakthrough discoveries of the highly energetic but non-damaging direct treatment of living tissues with non-thermal plasma enable to create new branch of the engineering medicine, PLASMA MEDICINE, which creates qualitatively new possibilities of healing, treating of previously untreated diseases, deactivation of dangerous pathogenic organisms, development of new direct methods of medical diagnostics. New types of non-thermal atmospheric plasma discharges are able to operate directly contacting human body and other living tissues, which significantly increase effectiveness of the tissue sterilization, treatment of wounds, skin and other diseases, as well as direct medical diagnostics. Obviously success of the plasma medicine depends on deep fundamental understanding of physics, chemistry and biology of the non-thermal plasma interaction with living tissues, and engineering of the relevant non-thermal plasma discharges, which is to be discussed in the presentation. Recent achievements in plasma biotechnology also address many aspects of the challenging problem of deactivation of viruses and bacteria that cannot be disinfected by traditional methods. Disinfecting large volumes of air in buildings and hospitals economically is now possible with room-temperature atmospheric pressure plasma. Similarly, atmospheric plasma technology can be employed to sterilize medical equipment, clothing, and building walls; to disinfect living tissue without side effects, and to disinfect and preserve food and water without damage. In addition, plasma technology can also be used to create innovative tools for sensing, detection and identification of dangerous pathogenic organisms as well as to characterize success of the cleansing processes. Essential advantage of the plasma biotechnology is its potential for universal availability, due to the technology's exclusive reliance on electrical power. It avoids many logistical difficulties associated with delivery, storage and disposal that typically hinder chemical and pharmaceutical approaches to sustainable health. Plasma technology can also be easily scaled from point-of-use devices to centrally operated plants capable of cleaning massive quantities of material. The key element of recent plasma technology developments is its use as a catalyst of many natural biological processes. As such, plasma can provide highly energy efficient treatment of biological materials, which is also to be discussed in the presentation.

3:00pm **BO+PS+AS+BI+SS-TuA5 Plasma Polymer Patterning of PDMS for Microfluidic Application**, ***S. Forster***, *A.G. Pereira-Medrano, M. Salim, P.C. Wright, S.L. McArthur*, University of Sheffield, UK

Microfluidic systems are becoming increasingly important for a wide range of bioengineering applications including proteomics and protein separations. Polydimethylsiloxane (PDMS) has proved to be the most popular material for microfluidic device production in the laboratory due to its many advantages over traditional materials. However, PDMS has some fundamental problems, namely a lack of functionality present at the surface, high protein fouling and 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. Plasma polymerisation is a method of depositing a uniform polymeric coating onto a surface, while retaining the desired functionality of the monomer. Hence, plasma polymerisation presents a versatile approach for surface modification and patterning of device channels. The wide range of monomers available for plasma polymerisation makes this approach even more suitable for use in systems where multiple surface properties within a single device are required. The aim of this work was firstly to investigate methods to produce stable plasma polymer patterns on PDMS. The coatings chosen include acrylic acid and maleic anhydride for their functional groups and tetraglyme to reduce non-specific protein adsorption. Patterning using photolithographic techniques and subsequent specific biomolecule immobilisation was achieved. Surface characterization using XPS and ToF-SIMS was used to ensure the spatial, chemical and biomolecule resolution of the device surfaces produced. This ability to combine microfluidics with spatially defined reactive regions on a 'non-fouling' background was then used in a number of applications to show the diversity and efficiency of the devices. Protein digestion by immobilized trypsin using single flow-through experiments in PDMS devices was improved using plasma polymer functionalized channels. The results achieved using mass spectrometry showed an increase in speed and sensitivity of the digestion as well as superior device reliability. Finally, plasma functionalized channels were used to investigate the effect of ampholyte adsorption onto device walls in isoelectric focusing (IEF). By coating channels with a tetraglyme plasma polymer an increase in sensitivity and reproducibility of IEF measurement was achieved. This technique can also increase the 'lifetime' of the device by ensuring channel properties were unchanged.

4:00pm **BO+PS+AS+BI+SS-TuA8 Plasma Etching for Selective Removal of PMMA from nm-scale PS/PMMA Block Copolymers for Lithographic Applications**, ***A.E. Wendt***, *Y.H. Ting, C.C. Liu, X. Liu, H.Q. Jiang, F.J. Himpsel,* University of Wisconsin-Madison, *P.F. Nealey*, University of Wisconsin, Madison **INVITED**

Diblock copolymers films, in which polymer components segregate into nano-scale domains, have been shown to have tremendous potential in fabrication of nm-scale surface topographies. Applications range from microelectronics fabrication to the study of how topography affects the growth and behavior of living cells or microorganisms. Use of block copolymers as a template for pattern transfer requires selective removal of one polymer component, and has motivated our study of plasma etching of polystyrene (PS) and polymethyl-methacrylate (PMMA), the two components of the PS-PMMA diblock copolymer. To better understand the mechanisms of the etch process for these materials, we have surveyed the effects of etch gas mixture and ion bombardment energy (taking advantage of our capability to produce a narrow ion energy distribution at the substrate), in combination with chemical analysis of the resulting etched surfaces. Of particular interest are the mechanisms of surface roughening, which shows a complex dependence on plasma process conditions that is not easily explained. A review of the literature on factors contributing to surface roughness, such as intrinsic inhomogeneity in the film, local deposition/micro-masking, shadowing effects and redeposition will be presented. We ultimately propose a mechanism for roughening of PS that involves micro-masking by inhomogeneous modification of surface chemical composition (rather than deposition) in oxygen-containing plasmas. Support from the UW NSF MRSEC for Nanostructured Materials is gratefully acknowledged.

4:40pm **BO+PS+AS+BI+SS-TuA10 Correlation of Macroscopic Surface Qualities of Poly-Parylene with Plasma-Specific Parameters**, ***G. Franz***, *F. Rauter, M. Häge*, University of Applied Sciences, Germany

In the course of our research how deposition conditions teleologically influence the morphology and various physical properties of the surface of

various derivates of parylene, we followed the Yasuda approach to correlate the deposition rate of polymeric films with external parameters (flow rate and power) to define three different regimes of growth.[1,2] Since external parameters, especially the pressure, influence the polymerization in an opposite manner (rising the pressure causes an increase in the collision rate, but a decrease in electron temperature) we studied the deposition of parylene vapors with and without pulsed microwave plasmas to correlate outcome parameters such as surface energy, roughness, and deposition rate with respect to plasma density and electron temperature (Langmuir and OES) by varying the molar fraction of the monomeric species, diluted by the noble gas argon, the total pressure and the power. For this end, we determined the vapor pressure of the dimer and the chemical equilibrium between the monomer and the dimer by varying the evaporation temperature and the cracking temperature, resp., and cross-checked this equilibrium by mass spectrometry. This method has been extended to explain the onset of volume polymerization which becomes manifest by slight tarnishing of the polymer. Following Yasuda, this happens when a certain ratio of number density of the monomeric species to plasma density is exceeded. After having established stable process windows, two further tracks have been followed, namely copolymerization with CF_4 (volume polymerization) and hydrophilic functionalization. Following Gogolides, the surface roughness has been correlated to contact angle measurements.[3] The super-hydrophobic character is mainly due to surface roughening (nanotexturing) in the case of normal CVD. However, plasma treatment leads to super-hydrophobic character also for smooth surfaces. Subsequent treatment with O_2 generates long-term stable hydrophilic surfaces. To calibrate the effect of momentum transfer and to separate the chemical effect of etching, this has been compared with Ar etching.

[1] H.K. Yasuda, and Q.S. Yu; J. Vac. Sci. Technol. A 19, 773 (2001)
[2] Q. Yu, C.E. Moffitt, D.M. Wieliczka, and H. Yasuda; J. Vac. Sci. Technol. A 19, 2163 (2001)
[3] A.D. Tserepi, M.-E. Vlachopoulou, and E. Gogolides; Nanotechnology 17, 3977 (2006).

5:00pm **BO+PS+AS+BI+SS-TuA11 Plasma Processing of Nanostructured Polymeric Surfaces for the Development of Immunosensors**, *A. Valsesia, **P. Colpo**, I. Mannelli, G. Ceccone, F. Rossi*, European Commission Joint Research Centre, Italy

Immunosensors play a very important role for the development of Point-of-Care analysis thanks to their rapid and sensitive detection capabilities.[1] Among others, the control of the interface between the transducer and the biological probes is a crucial issue since the bio-interface is the essential element that guaranty the bioactivity of the immobilized biological probes.[2] The control of the bio-interface is typically addressed by functionalizing the surface with special chemical groups. Besides, new nanobiotechnology-based tools have led to more sophisticated approaches that use for instance nanostructured surfaces. Benefits have been already shown in terms of the improvement of immunoreaction efficiency.[3] In this work we propose a new method for fabricating nanostrucured surfaces combining the use of colloidal masks with different plasma processes. In this method, Plasma Polymerization Processes are able to produce pinhole-free functional layers with different properties. The choice of the precursor together with the appropriate plasma processing parameter ensures the production of stable functional layers which can be used for the production of the chemically contrasted nanopatterns. Also the deposition of the colloidal mask in a controlled way is essential: for example, mass sensitive detectors (like Quartz Crystal Microbalance, QCM) require the use of very large areas in order to obtain measurable signals. Also plasma etching plays a very important role: it is important to choose the suitable processing parameters enabling the fabrication of nanostructured surface which are not limited in the patterning geometry and resolution. After the optimization of the nanofabrication process, the surfaces of immunosensors have been nanostructured. In particular we transferred the nanostructures on the crystals of QCM for on-line monitoring of the protein adhesion. The nanostructures accelerate the kinetics of absorption and increase the density of absorbed molecules, resulting in higher bioactivity of the immobilized proteins and consequently in an improvement of the immunosensing performances.

[1] K. R. Rogers, Applied Biochemistry and Biotechnology - Part B Molecular Biotechnology 2000, 14, 109-129.
[2] B. Kasemo, Current Opinion in Solid State and Materials Science 1998, 3, 451-459.
[3] A. Valsesia, P. Colpo, T. Meziani, P. Lisboa, M. Lejeune, and F. Rossi, Langmuir 2006, 22, 1763-1767.

5:20pm **BO+PS+AS+BI+SS-TuA12 Use of Multivariate Analysis Techniques to Predict Cellular Response to Plasma Polymerized pNIPAM**, ***J.E. Fulghum**, K. Artyushkova, A. Lucero, H.E. Canavan*, University of New Mexico

The primary objective of this work is to investigate the correlate structural properties of a thermoresponsive polymer, poly(N-isopropyl acrylamide) (pNIPAM), with its ability to reversibly adhere cells. PNIPAM undergoes a sharp property change in response to a moderate thermal stimulus at physiological temperatures (~32 °C). This behavior has generated great interest in the biomaterials community, and pNIPAM is being investigated as a "smart" release coating to harvest intact cell monolayers. Many techniques are used to deposit pNIPAM, including electron beam irradiation and solution deposition (e.g., silanes and self-assembled monomers). Recently, we constructed a radio frequence (rf) plasma reactor for plasma polymerization of NIPAM (ppNIPAM) from the vapor phase based on a previous design. Plasma polymerization is a sterile, solvent-free, and compatible with surfaces of any geometry or chemistry. These factors make plasma polymerization extremely useful for cell and tissue culture, which often rely on plastic tissue culture plates. Due to the inherently energetic conditions of the plasma, parameters such as maximum rf wattage, location/position of the samples in the chamber, and monomer flow have on the resulting films. In this work, pNIPAM films resulting from those varying conditions are characterized using X-ray photoelectron spectroscopy (XPS) for film composition, interferometry for film thickness, contact angles for thermoresponse, and cell detachment for cell releasing properties. Using multivariate analysis, the structural information of the films obtained at various polymerization conditions will be correlated with their thermoresponsive and cell-releasing behavior. In this way, we will predict the conditions that will optimize film composition for bioengineering applications.

Biomaterial Interfaces
Room: Hall D - Session BI-TuP

Biomaterials Interfaces Poster Session with Focus on Cells and Proteins at Interfaces

BI-TuP1 Human Serum Albumin Adsorption to Expanded Polytetrafluoroethylene, ***E.S. Leibner***, *N. Barnthip, W. Chen, C.R. Baumrucker, J.V. Badding,* The Pennsylvania State University, *M.V. Pishko,* Texas A&M University, *E.A. Vogler*, The Pennsylvania State University

Analytical protocol greatly influences measurement of human-serum albumin (HSA) adsorption to commercial expanded polytetrafluororethylene (ePTFE) exhibiting superhydrophobic wetting properties (water contact angles in excess of 150°). Degassing of buffer solutions and evacuation of ePTFE adsorbent to remove trapped air immediately prior to contact with protein solutions are shown to be essential steps that assure reproducible measurement of HSA adsorption. Results obtained with ePTFE as a superhydrophobic test material suggest that vacuum degassing should be generally applied in the measurement of protein adsorption to any surface exhibiting superhydrophobicity. Solution depletion quantified using radiometry (I-125 labeled HSA) or electrophoresis (unlabeled HSA) yield different measures of HSA adsorption, with nearly seven-fold higher surface concentrations of unlabeled HSA measured by the electrophoresis method. This outcome is attributed to the influence of the radiolabel on HSA hydrophilicity which decreases radiolabeled-HSA affinity for a hydrophobic adsorbent in comparison to unlabeled HSA. These results indicate that radiometry underestimates the actual amount of protein adsorbed to a particular material. Removal of radiolabeled HSA adsorbed to ePTFE by 3X serial buffer rinses also shows that the remaining "bound fraction" was about seven-fold lower than the amount measured by radiometric depletion. This observation implies that measurement of protein bound after surface rinsing significantly underestimates the actual amount of protein concentrated by adsorption into the surface region of a protein-contacting material.

BI-TuP2 The Role of Surface Chemistry in poly-L-lysine Mediated Alginate Adsorption, ***K.T. Queeney***, *J.E. Brown, E.K. Davis, C. Franqui*, Smith College

Adsorption of polysaccharides to solid substrates, an essential component of biofilm formation, is generally protein-mediated. Lysine-rich proteins, in particular, are known to have significant adhesive properties in these systems. This work uses poly-L-lysine (PLL) as a model protein to explore the role of the underlying substrate in protein-mediated adsorption of alginate, a negatively-charged polysaccharide. We have prepared films of varying PLL coverage on two types of surfaces: hydrophilic, negatively-charged silica and a hydrophobic, electrically neutral alkylsilane monolayer. PLL coverage on the hydrophilic surface is controlled by varying the ionic strength of the adsorption solution, while PLL coverage on the hydrophobic surface is controlled by varying the molecular weight of the PLL. By a combination of techniques--primarily ellipsometry, dynamic contact angle goniometry and atomic force microscopy--we demonstrate that not only the film morphology of the PLL but also its effect on subsequent alginate adsorption is highly sensitive to the surface chemistry of the underlying substrate. For example, PLL films on silica are more discontinuous at low and high ionic strength, and alginate adsorption is highly influenced by this discontinuity, which allows for repulsive electrostatic interactions between the surface and the alginate. In contrast, while there is an increase in PLL film discontinuity as a function of increasing PLL molecular weight, alginate adsorption appears largely insensitive to these changes in PLL film morphology. Through quantitative analysis of the advancing contact angles on both these (chemically heterogeneous) surfaces we demonstrate unequivocally that the PLL/solution interface is highly dependent on both substrate surface chemistry and PLL adsorption conditions.

BI-TuP3 Time of Flight Secondary Ion Mass Spectrometry Characterization of the Surface Polysaccharides of Group B Streptococcus, *T. Nguyen,* Oregon State University, ***J.R. Hull***, *D.G. Castner*, University of Washington NESAC/BIO

Group B Streptococcus (GBS) is a leading cause of sepsis and meningitis in neonates and immunocompromised adults in western countries. The surface of GBS is well characterized by standard microbiological techniques and therefore makes a good test system for analyzing bacteria using Time of Flight Secondary Ion Mass Spectrometry. GBS is layered with a capsule composed of five distinct polysaccharides containing glucose, galactose, N-acetylneuraminic acid, rhamnose, and N-acetylglucosamine. The capsule makes up 10 to 30% of the dry weight of the microorganism and is only present on the surface. In this work, the five monosaccharides, pure capsule from type III GBS, and UV killed GBS strain COH1 and acapsular GBS strain COH1-13 were investigated. It was observed that the pure monosaccharide fragmentation patterns followed $C_xH_{2x}O_x^+$, and that the largest fragment observed of the monosaccharides was with the loss of H_2O, OH^- or H_3O^+. The presence of sodium, magnesium, and calcium in the purified samples made direct comparison with the pure monosaccharides difficult due to cationization effects. Spectra from clusters of GBS and single organisms were acquired using the high mass resolution imaging mode and constructing a spectrum from a region of interest. The biggest differences between COH1 and COH13 were seen in the high mass region of the spectra.

BI-TuP4 Hydrogels with KGF, ***J.A. Appawu***, *J.A. Gardella*, University at Buffalo: The State University of New York

XPS and TOF-SIMS are techniques that can be used to quantify the uptake and release of proteins from polymers. Hydrogels are cross-linked hydrophilic polymers used in localized delivery of growth factors through pores defined by type and density of cross-links. Therefore uptake and release are diffusion controlled. Keratinocyte growth factor 1(KGF-1) is known to promote re-epithelialization after skin injury and stimulate the proliferation of skin cells. Inadequate amounts of growth factors can result in inefficient healing. In this study, (hydroxyethyl methacrylate) (HEMA) hydrogels 3% and 6% were prepared with varying cross-link densities to answer three key questions:1) What is the optimal concentration of KGF-1 for cell adhesion, 2) Are HEMA hydrogels with specific structural properties viable for cell proliferation, and 3) What is the amount and time course of released KGF-1 present on the surface? Hydrogels were cut into equal areas to determine the amount of KGF-1 protein taken into and released from the hydrogels by fluorescence spectroscopy. XPS was used to confirm the quantity of KGF-1 on the surface and bulk by varying the take-off angle (TOA). The nitrogen atomic concentration was used to track the protein since nitrogen is present only in KGF-1. The amount of KGF-1 was higher in the 3% hydrogel due to a swelling ratio of 58.5% compared to 40.5% for the 6% hydrogel. Cell adhesion experiments have shown that 80-90% of HaCaT cells successfully adhere to the surface of the KGF-1 imbibed hydrogels and fluorescence microscopy proved that the cells were alive. TOF-SIMS has been used in depth profiling to confirm the XPS results.

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BI-TuP5 Patterning of pOEGMA Polymer Brushes Using Photolithography and Photomasking for Applications in Protein and Cellular Adhesion Research, *M.S. Johannes, A. Garcia,* ***A. Hucknall***, *A.J. Simnick, R.L. Clark, S. Zauscher, A. Chilkoti*, Duke University

We report the use of bulk microfabrication processes to pattern non-fouling, surface-initiated poly-oligoethylene glycol methacrylate (pOEGMA) polymer brushes. Previous studies have demonstrated the non-fouling capability of pOEGMA surfaces through protein absorption and cell adhesion studies. Here, we demonstrate that the polymer brushes on a glass substrate can be easily patterned using two processes. In the first one, standard photolithography is used to pattern both negative and positive photoresist spin-coated on the pOEGMA layers. The patterned photoresist layers act as masks for the removal of exposed underlying pOEGMA layers by UV-O3 exposure and oxygen plasma ashing. Upon photoresist stripping, patterns with a resolution down to ~400 nm are achieved. In the second approach, TEM grids are directly placed on the pOEGMA layer to block the brushes from direct exposure to various oxidative species offering great simplicity, high-throughput, and low-cost. Successful patterning of non-fouling surfaces at both the micron and nanometer scale will have importance in developing patterned cell monolayers and studying linear motor protein locomotion.

BI-TuP6 Novel Patterned Protein Assay To Measure Differential Extracellular Matrix Protein Affinities for Cellular Attachment and Axonal Outgrowth, ***W.M. Theilacker***, *A.L. Styer, H.P. Bui,* University of Delaware, *D.E. Willis, J.L. Twiss,* Alfred I. DuPont Hospital for Children, *T.P. Beebe, Jr.*, University of Delaware

Cellular preference for extracellular matrix (ECM) proteins was assayed on patterned surfaces presenting two ECM proteins that compete for cell attachment and proliferation. Microcontact printing techniques were used to modify silicon substrates with alternating 40-um-wide stripes of the ECM proteins fibronectin and laminin. The spatial distribution of both proteins on the patterned surfaces was measured by epi-fluorescence and Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS). Attachment and outgrowth of dorsal root ganglia (DRG) neurons and neuron-like pheochromocytoma (PC12) cells on striped substrates were analyzed up to 4 days. At each time point, three patterned samples were fixed and immunostained prior to fluorescence imaging. Images were analyzed for the number of cells attached to each protein region and the number and length of neurite extensions. Results indicate for PC12 cells, an approximately equal number of cells on fibronectin and laminin stripes after 24 hours in cell culture. However, from 48 hours to 96 hours, the number of cells on laminin versus fibronectin continually increased. By 96 hours, 80 percent of the PC12 cells were attached to laminin versus fibronectin. Preliminary results for DRG neurons suggest a similar trend, in addition to the influence of Schwann cells, which are known to influence DRG neurite outgrowth.

BI-TuP7 XPS Analysis of Protein Adsorption on Bioprocessing Materials, ***R.A. Zangmeister***, National Institute of Standards and Technology

Protein therapeutics, a fast-growing category of biotech drugs, is adversely affected by protein aggregation. Aggregation can decrease the safety and efficacy of the therapeutic biologic protein product. Currently, the cause of protein aggregation is not clearly understood. The adsorption of protein therapeutics to bioprocessing materials used in production, storage and delivery may create nucleation sites for further protein aggregation. X-ray photoelectron spectroscopy was used to analyze protein adsorption on bioprocessing materials to screen for those with increased probability of contributing to aggregation of the protein therapeutic. The standard overlayer model and protein labeling were used to estimate the thickness of adsorbed protein layers.

BI-TuP8 Highly Sensitive Probe of Crystalline Sub-Micron Sized Domain Structures in Amorphous Material, ***H.B. de Aguiar***, *S. Roke*, Max-Planck-Instiut fuer Metallforschung, Germany

Mixtures of solid compounds or phases occur in a wide variety of chemical processes. Nucleation, crystallization and separation of phases are generally integrative steps in the production of polymer compounds, pharmaceuticals and in many other chemical processes. Many techniques used for analyzing mixtures of medium and ingredients in-situ rely on the interaction of photons with the mixture.[1] Such a situation occurs in solid/solid dispersions, where two different phases coexist, as in the case of most polymers which are known to consist of crystalline domains embedded in an amorphous phase. For instance, Wide-Angle X-Ray Diffraction (WAXS) and Small-Angle X-Ray Scattering (SAXS) are used together to obtain crystallite structure, size and fraction, being limited only by the level of background signal relative to the signal itself. We developed a new technique to study crystalline domains dispersed in an amorphous phase through nonlinear optical Vibrational Sum-Frequency Generation (VSFG) Scattering.[2] VSFG is a second-order nonlinear optical process, which is forbidden in centrosymmetric media (e.g. liquids, amorphous solids). We show the higher sensitivity of VSFG scattering to microspheres (MS) consisted of crystalline and amorphous phase. We studied the scattering pattern of some chiral polymers, namely Poly(Lactic Acid) (PLA). MS made of P(DL)LA polymer (polymer chains consisting of a random distribution of enantiometers) were probed by X-Ray Diffraction (XRD) and Differential Scanning Calorimetry (DSC) as well. XRD and DSC showed no crystallinity of the PDLLA MS, in agreement with previously published studies. However, VSFG spectra and scattering patterns demonstrate that there is still a finite number of crystallites with maximum extension of 250 nm (radii). This indicates VSFG has an increase in sensitivity that is roughly 2 orders of magnitude more than XRD.[3] This opens up new possibilities in the description of early stages of nucleation and growth phenomena and possible new phases.

[1]S Roke et al. Vibrational Sum Frequency Scattering from a Submicron Suspension. Phys. Rev. Lett. 91, 2003, 258302.

[2]AGF de Beer et al. Molecular and microscopic properties of buried microstructures. Submitted.

[3]HB de Aguiar and S Roke. Sum-Frequency Generation Scattering: a higher sensitivity probe for crystalline properties. In preparation.

BI-TuP9 Effect of Film Fabrication Method on the Protein Fouling Resistance of Mixed Oligo(ethylene glycol) Films, ***J.A. Ruemmele***, *M.S. Golden, R.M. Georgiadis*, Boston University

A major concern in the design of biosensors for studying protein interactions is protein fouling, or non-specific binding, which masks the signal from the binding event of interest. Mixed oligo(ethylene glycol) (OEG) films have become a standard surface coating used to avoid fouling of gold sensor surfaces. Such films comprise a probe coupling OEG (A) and a diluent of protein resistant OEG (B). Two methods exist for the fabrication of such surfaces: co-deposition of A and B, and sequential deposition, where A is inserted into a pre-existing monolayer of B. Though films fabricated by sequential deposition display better fouling resistance, the source of this resistance is unclear. It has been hypothesized that the improved fouling resistance is due to an absence of islanding in the film, however it could simply result from an extremely low A content. In order to identify the affect of the fabrication method, films prepared by both approaches must contain the same amount of A. Since few techniques exist which can accurately measure low amounts of A attached to a surface, the amount of probe which covalently couples to a film was used to evaluate the film's A content. Using this technique, the fabrication conditions for both methods were adjusted to produce films containing the same amount of A. The extent of fibrinogen fouling on these now comparable films was measured with surface plasmon resonance imaging to evaluate if the actual fabrication method affects a film's fouling resistance. Furthermore, electrochemically induced reductive desorption was used to indicate any presence of islanding and to evaluate film stability.

BI-TuP10 Specific versus Non-Specific Protein Adsorption: Effects of Chain Length and Tailgroup in Functionalized Poly(ethylene glycol)-Terminated Self-Assembled Monolayers, *P. Buecker, M. Himmelhaus,* ***R. Dahint***, University of Heidelberg, Germany

In recent years, substantial efforts have been made to develop surface coatings which limit or even suppress non-specific adsorption of proteins. While for many technological applications the surfaces are designed to exclusively repel proteins, an even more complex situation exists in biomedical analysis, where immobilized probe molecules, e.g. antibodies, are used to specifically bind target proteins from solution. Here, the coating must fulfil a two-fold function: (i) effectively suppress non-specific adsorption processes, which may both result in false interpretation of binding events and significantly lower the detection limit of the analytical techniques, and (ii) additionally facilitate the integration of the probe molecules without loss of non-specific protein repulsion. Hereby the question arises, to what extent efficient immobilization of probe molecules and suppression of non-specific interactions can actually be achieved with a single material as the repulsion mechanism inhibiting non-specific adsorption of biomolecules might also compromise the integration of the probes. In a recent study we have shown that COOH-functionalized poly(ethylene glycol) (PEG) alkanethiolate self-assembled monolayers (SAMs) [$HS-(CH_2)_{11}-(OCH_2CH_2)_n-COOH$] with a mean number, n, of 33 EG units suppress non-specific protein adsorption while facilitating covalent coupling of antibodies via the terminal COOH-group.[1] We now synthesized the same type of molecule with various EG chain lengths (n = 13-40) and different tailgroups (-OH, $-NH_2$, and -COOH), and compared both the antigen binding capacity and the protein resistance of the corresponding SAMs by ellipsometry, X-ray photoelectron spectroscopy (XPS), infrared spectroscopy (FT-IRRAS), and enzyme-linked immunosorbent assays (ELISA). It is observed that protein repulsion depends on the tailgroup selected and increases with increasing EG chain length. In parallel, however, the maximum amount of probe molecules that can be coupled to the films significantly decreases with enhanced EG content, thus, reducing the antigen binding capacity of the films. The results, therefore, show that for bioanalytical applications the number of EG units has to be properly adjusted in order to obtain an optimum signal-to-noise-ratio. The best performance has been observed for a chain length of about 30 EG moieties.

[1] S. Herrwerth et al., Langmuir 2003, 19, 1880.

BI-TuP11 Quantitative Analysis of Angular Resolved XPS-Measurements: Influence of Inaccuracies in Take-Off and Acceptance Angle, ***C. Van der Marel***, *J.H.M. Snijders, D.D.C.A. Van Oers, E.P. Naburgh*, Philips Research - MiPlaza, The Netherlands

For quantitative analysis of Angular Resolved XPS-measurements the take-off angle of the detected electrons is an important input parameter. A device has been developed that allows measurement of the real take-off angle as a function of the tilt angle of the sample. The acceptance angle of the detector is also determined using this device. The device has been tested extensively in two commercial XPS-instruments (Quantera from ULVAC – PHI); small but significant deviations have been found between real and set values of the take-off angles. To investigate the influence of the acceptance angle and of inaccuracies in the take-off angles several series of samples were

analyzed. The samples consisted of a homogeneous substrate coated with a thin organic layer (e.g. phosphonic SAM-layers on Al2O3, alkyl-thiols on copper). Quantitative analysis of the XPS-results provided values for the layer thickness of the organic layer, for the coverage and for the "real" atomic concentrations in substrate and in the organic layer. In the lecture it will be shown that the use of accurate values of the acceptance and the take-off angles is a prerequisite to obtain consistent results.

BI-TuP12 Improved Osseointegration of a Novel, Hydrophilic Ti Surface: Early Events, *A. Molenberg*, ***S. Berner***, Institut Straumann AG, Switzerland, *F. Schwarz, M. Herten,* Heinrich Heine University, Germany, *M. de Wild, M. Wieland*, Institut Straumann AG, Switzerland

The performance of bone-related implants is strongly connected with the properties of the implant surface. Particularly surface topography and chemistry both influence initial wettability and peri-implant bone apposition of implants. Sandblasted and acid etched (SLA) titanium surfaces are initially hydrophobic due to microstructuring and adsorption of hydrocarbons and carbonates. The purpose of this study was to investigate initial hydrophilicity and to examine tissue reactions to a newly developed chemically modified implant surface called SLActive. Implantation of SLActive and SLA implants was performed bilaterally in dogs. The animals were sacrificed after a healing period of 1, 4, 7 and 14 days, respectively. Peri-implant tissue reactions were assessed in nondecalcified tissue sections using conventional histology (Toluidine blue-TB and Masson Goldner Trichrome stain-MG) and immuno¬histochemistry using monoclonal antibodies to transglutaminase II (TG) (angiogenesis) and osteocalcin (OC) (mineralization). Bone density (BD) and bone to implant contact (BIC) were assessed histomorphometrically. Immunohistochemical staining for TG revealed a direct correlation between angiogenesis and new bone formation, which was clearly identifiable after 7 days by means of increasing BD, BIC and OC values. After 14 days, SLActive surfaces seemed to be surrounded by a firmly attached mature, parallel-fibered woven bone. The study clearly reveals an enhanced bone formation for the hydrophilic SLActive surface and thus a reduced healing time.

BI-TuP13 Solution Deposited poly(N-isopropyl acrylamide) Films Optimized for Mammalian Cell Release, ***J.A. Reed***, *M. Bore, L.K. Ista, G.P. Lopez, H.E. Canavan*, University of New Mexico

Poly(N-isopropyl acrylamide), or pNIPAM, is a smart polymer that is of great interest in the bioengineering community. Above its lower critical solution temperature (LCST), the 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 hydrophilic and swelling. Furthermore, when the temperature of the cell culture is dropped below the LCST of the polymer, the cells will detach and can be harvested for tissue engineering applications. This is in contrast to cells grown on TCPS alone, which will not detach using a temperature drop, instead requiring enzymatic digestion (via trypsin) or mechanical scraping to remove them. In this work, we present a novel, solution-based technique to generate pNIPAM surfaces from pNIPAM and tetraethyl orthosilicate (spNIPAM). The composition of the films (i.e., weight % of pNIPAM) was optimized for reversible cell adhesion by observing the thermoresponse, surface chemistry, and cell release characteristics. Characterization of the surface chemistry of the spNIPAM substrates was done using X-ray photoelectron spectroscopy (XPS), interferometry, and contact angle measurements). Characterization of the cell-releasing behavior of the spNIPAM substrates was determined by observing the ability of surfaces to release confluent bovine aortic endothelial cells (BAECs). From these results, we conclude that the fastest, most reliable release of BAECs occurred on surfaces spin coated with a solution of .35wt% pNIPAM/TEOS.

BI-TuP14 Nanometer-scale Cell Adhesive Patches for the Control of Cellular Functions, ***U.A. Martinez***, *T.C. Gamble, G.P. Lopez, E.L. Dirk*, The University of New Mexico

The control of cellular functions will find many applications in the development of new materials for biomedical applications such as tissue engineering. However, for this to be possible, understanding the cell-material interactions is essential. In this work, interferometric lithography was used to create nanometer-scale patterned substrates. Interferometric lithography is a method for fabricating spatially consistent periodic patterns with the use of the interference of two coherent optical beams. The interfering beams produce a standing wave that can be recorded in a photoresist making equally spaced channels. The substrate is then rotated 90° and exposed again creating photoresist pillars. After exposure, the substrates are coated with a 30 nm layer of gold, using a 3 nm layer of chromium to enhance adhesion. An acetone lift-off procedure removes the photoresist pillars, leaving uncoated patches throughout the gold-coated surface. Self-assembled monolayers are formed on the gold-coated areas using thiols of poly(ethylene glycol) [PEG], a biologically inactive molecule that inhibits protein adsorption. Fibronectin, a protein that enhances cellular adhesion, is adsorbed onto the uncoated regions rendering cell adhesive patches. A double exposure technique has also been developed to obtain a two-fold increase in the pitch while holding the same patch size. For example, a pitch of 300 nm with a 100 nm patch can be increased to a 600 nm pitch while maintaining the same size of the patch. These nanopatterned cell adhesive substrates offer a method to systematically manipulate cellular attachment to further control cellular functions such as spreading, viability and differentiation.

BI-TuP15 Competitive Protein Adsorption to Biomaterials and the Dependence on Protein Concentration, ***M. Holmberg***, *X. Hou*, Technical University of Denmark

Quantitative analysis of protein adsorption to artificial materials is of importance in evaluating the potential of biomaterials. Proteins have a central role in all living organisms and are present in basically all parts of the human body and the first thing to happen when an artificial material is introduced into a human body, or another biological system, is unspecific adsorption of proteins onto the surface of the material. Most studies on blood protein adsorption to polymer surfaces reported in the literature are performed from low concentration (≤1 mg/ml) single protein solutions. However, it is not evident that those results also are valid for protein adsorption from protein mixtures with high protein concentration (≥10 mg/ml). When biomaterials are introduced into a human body, thousands of different proteins, sometimes present in quite high concentrations, will come in contact with the material and there will be competitive protein adsorption on the surface of the material. By labelling proteins with different radioisotopes one can monitor the adsorption of several proteins onto surfaces simultaneously, and with this multi-labelling technique one can study competitive adsorption and use complex solutions such as human serum during adsorption experiments. Albumin and IgG (Immunoglobulin G) adsorption to both unmodified and modified PET (polyethylene terephthalate) surfaces from single protein solutions and human serum solutions has been monitored using radioactive labelling. The PET surfaces are modified through plasma polymerisation using the monomer DEGVE (diethylene glycol vinyl ether), which results in a more hydrophilic and anti-fouling surface than the PET surfaces. Results show that the detected anti-fouling quality of DEGVE surfaces is influenced by the concentration of the protein solution used. Albumin and IgG adsorption from human serum dilutions corresponding to 0.1% human serum shows that the albumin and IgG adsorption is significant lower on the DEGVE surface than on the PET surface, and that this antifouling characteristics of DEGVE surfaces can be stable for at least 24 hours. However, performing the same experiment, including the same rinsing procedure, with ≥10% human serum solutions, the detected albumin and IgG adsorption to DEGVE approaches the adsorption detected on unmodified PET. Thus, evaluation of biomaterials should be performed in an environment similar to the one the material is intended to operate in.

BI-TuP16 Scanning Tunneling Microscopy Studies of Photoactive Yellow Protein Anchored to the Thiol Modified Au(111) Surface, ***I.I. Rzeznicka***, RIKEN, Japan, *G.W.H. Wurpel*, Utrecht University, The Netherlands, *M. Bonn*, FOM Institute for Atomic and Molecular Physics (AMOLF), The Netherlands, *M.A. van der Horst, K. Hellingwerf*, University of Amsterdam, The Netherlands, *S. Matsunaga*, The University of Tokyo, Japan, *T. Yamada*, RIKEN, Japan, *M. Kawai*, The University of Tokyo, Japan

Adsorption of proteins on metal surfaces and their interactions present an important technological challenge in the field of biotechnology and construction of biomolecules-based functional devices. The process of protein adsorption is very complex and depends on surface properties, protein structure and its chemical composition. On Au surfaces, covalent bonding to the surface can in principle be easily achieved by utilizing unbound sulfur-containing amino acids resulting in the formation of strong Au-S bond, in analogy to the well known thiol chemistry. We studied adsorption of photoactive yellow protein (PYP) protein on Au(111) surface by scanning tunneling microscopy (STM) in air and in the electrochemical environment. The results show that cysteine-containing mutant of PYP does not adsorb on the bare Au(111) surface. Stable, densely packed protein layers were observed after protein immobilization via amide bond formation onto self-assembled monolayers (SAMs), consisting of 3-mercaptopropanoic acid (3-MPA) and 11-mercaptoudecanoic acid (11-MUA) with the use of carbodiimide and N-hydroxysuccinimide. Layers of PYP prepared in this way show fluorescence activity visualized by fluorescence microscopy, indicating that the protein retains its photoactive function upon adsorption. We will also present our attempts to detect a photocurrent generated by laser illumination of PYP layers.

BI-TuP17 Fabrication of Micro-Templates for the Control of Bacterial Immobilization, ***Y. Miyahara***, *N. Saito, O. Takai*, Nagoya University, Japan

Well-defined micro-patterns of bacteria are significant as a fundamental technique for biosensor arrays utilizing rapid detection of infectious diseases and toxic compounds. Many researchers have studied control methods to fabricate the micro-patterns. However, in these researches, many processes are required for the immobilization and the regioselectivity is not so good. In this study, we aim to fabricate well-defined micro-patterns of bacteria using two types of micro-patterned templates and to elucidate the adhesion behaviors of several bacteria onto the templates from viewpoint of surface topology and chemical properties. Super-hydrophobic/super-hydrophilic and super-hydrophobic/polyethylene glycol (PEG) micro-patterns were fabricated as the templates. In the case of super-hydrophobic/super-hydrophilic patterns, the difference of surface energy in the respective regions differs greatly. The difference could have a great effect on the adhesion of bacterial. In the case of super-hydrophobic/PEG micro-patterns, PEG surface generally avoid the adhesion of bacteria due to volume exclusion effect of PEG based on the structural fluctuation. Super-hydrophobic surface was prepared by microwave plasma enhanced chemical vapor deposition (MPECVD) from trimethylmethoxysilane (TMMOS). Super-hydrophobic/super-hydrophilic micro-patterns were fabricated by irradiating the super-hydrophobic surface with vacuum ultra violet (VUV) light through a stencil mask. In the case of Super-Hydrophobic/PEG micro-patterned surfaces, PEG surfaces were fabricated by reacting COOH groups of methoxypolyethylene glycol propionic acid with NH_2 groups of NH_2-terminated self assembled monolayer in ion-exchanged water. The super-hydrophobic regions were fabricated by MPECVD thorough a stencil mask. Bacteria were cultured on the respective templates in the incubator controlled under the CO_2 concentaration of 5 % at 37 ^{o}C. After culture, bacteria were observed by phase-contrast microscope. The surfaces were characterized by XPS and FT-IR. As a result, in the case of super-hydrophobic/super-hydrophilic micro-patterns, some bacteria (Escherichia coli, Bacillus subtilis) were adhered to only super-hydrophobic regions. But others bacteria (Pseudomonas stutzeri, Pseudomonas aeruginosa) were not separated well. Their results attribute bacterial charge, cell division rate and adhesion time lag between super-hydrophobic regions and super-hydrophilic regions.

BI-TuP18 Kinetic Study on Protein Adsorption on Polyelectrolyte Brush Surface, ***H. Tatematsu***, Nagoya University, Japan, *T. Fujima*, Musashi Institute of Technology, Japan, *N. Saito, O. Takai*, Nagoya University, Japan

Polymer chains grafted in the high density stretch perpendicularly by repulsive interaction. This is a so-called brush. The various functional groups can be also introduced into polymer brush as lateral chains. The 3-dimensional structure was varied by the type of lateral chains. On spherical polyelectrolyte brush (PEB), strong adsorption of protein takes place at low ionic strength whereas less protein is adsorbed at the high ionic strength.[1] In addition, protein structure and character remain via adsorption.[2] These properties can be applied to improvement of biocompatibility and Drug Delivery System (DDS). However, the kinetics of adsorption on normal PEB surface has not been understood well. Thus, in this study, we aimed to investigate the kinetics of protein adsorption on PEB. Polystyrene brush (PSB) surface was fabricated by reacting polystyrene terminated by a trichlorosilane (PS-Cl_3) group with silanol (Si-OH) groups on the silicon wafer. PSB brush was converted to polystyrene sulfonate sodium salt brush (PSSB) by sulfonation and neutralization. In protein adsorption experiment, the solution of Bovine Serum Albumine (BSA) in phosphate buffer saline (PBS) was used. NaCl aqueous solution adjusted to various concentrations was added to the stock solution in order to extract the effect of ionic strength. Protein adsorption was evaluated by Fourier transform infrared spectroscopy (FT-IR) and the evanescent adsorption spectroscopy (EOS). The proteins on the surfaces were observed by atomic force microscope (AFM). Relationship between the amount of adsorption and the ion strength on normal PSSB surface differs from that on spherical PSSB surface in previous reports. This would originate from structural configuration of PEB. Finally, we propose a kinetic model of the adsorption on PSSB based on the variation of adsorption against time and ion strength by FT-IR, EOS and AFM.

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BI-TuP19 Surface Characterization of PEG-like Film Made by Using Ethylene Glycol Precursor and Capacitively Coupled Plasma Chemical Vapor Deposition, ***C. Choi***, *S. Lee, D. Jung,* Sungkyunkwan University, Korea, *D.W. Moon, T.G. Lee*, Korea Research Institute of Standards and Science (KRISS)

Polyethylene-glycol (PEG) is widely used for various biological applications because of its anti-fouling property for protein and cells, and non-toxicity in the human body. Since the PEG thin film should have the properties of reproducibility and adhesiveness on various substrates, in this work, plasma-polymerized ethylene glycol (PPEG) thin film was deposited on a glass surface by using the capacitively coupled plasma chemical vapor deposition (CCP-CVD) method and ethylene glycol as a precursor. The PPEG thin films were characterized by using contact angle measurement, X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) along with a principal component analysis (PCA). The PPEG surface was controlled in a reproducible manner as a function of sample bias plasma power and was correlated with the surface chemical composition. Of interest, we found that PPEG thin film surfaces deposited at low plasma power were similar in chemical composition to the PEG polymer surface. In addition, by using the fluorescence detection method, we found that the PPEG surface showed an anti-fouling property of immunoglobulin G protein, which was tagged by fluorescein isothiocyanate. Our results showed that this PEG-like PPEG surface would be useful for protein chip applications.

BI-TuP20 Determination of Standard-State Adsorption Free Energy for Peptide-Surface Interactions by SPR Spectroscopy without Peptide-Peptide Interaction Effects, ***R.A. Latour***, *Y. Wei*, Clemson University

As an approach to gaining fundamental insights into the thermodynamics of protein-surface interactions, we are quantitatively determining the standard state free energy of peptide-surface interactions using a host-guest peptide model in the form of TGTG-X-GTGT, where the T (threonine) and G (glycine) flanking sequences are the host residues and X represents a variable guest residue. Alkanethiol self-assembled monolayers (SAMs) with a broad range of polymer-like functionalities are being used as the adsorbent surfaces. With this experimental model, we are able to determine contributions of individual mid-chain amino acid residues on peptide adsorption behavior. The most common way of determining the standard state free energy of adsorption of a peptide to a surface is by use of the Langmuir equation fitted to an adsorption isotherm. This method, however, has the inherent problem of the influence of peptide-peptide interactions at the interface. These effects can substantially affect the shape of the isotherm, leading to errors in the calculated values of the standard state free energy of adsorption. We have developed a new approach to solve this problem and provide accurate, quantitative measurements of the standard state adsorption free energy using surface plasmon resonance (SPR) spectroscopy based on chemical potential relationships vs. the classical Langmuir equation. In addition, we have also developed a very simple, automated method of accounting for bulk shift effects, which can be problematic when doing SPR adsorption studies. These novel methods will be presented along with results for a series of peptide adsorption systems using these methods.

Biomaterial Interfaces
Room: 202 - Session BI+NC-WeM

Quantitative Nanoscale Sensing and Single Molecule Techniques

Moderator: B. Liedberg, Linköping University, Sweden

8:00am **BI+NC-WeM1 Studying Single Molecules on Living Cells**, ***D. Klenerman***, Cambridge University, UK **INVITED**

One major challenge in biology is to understand how the individual molecules and complexes of the cell are organised and interact to form a functional living cell. To address this problem new biophysical tools are needed that are capable of studying single molecules in complexes both in the test-tube and on or in living cells. To determine the oligomerisation state of proteins we have used two colour single molecule coincidence detection based on the excitation of two distinct fluorophore labels on proteins with two lasers focussed to the same spot.[1] This method requires no prior knowledge of the structure of any complex formed or control of fluorophore position on the molecule. We show that this method can be used to characterise the protein oligomers formed during protein misfolding, ultimately resulting in amyloid fibril formation, and can distinguish between protein monomers and dimers on the cell surface.[2] Working together with Professor Yuri Korchev at Imperial College, we have developed a method for functional nanoscale mapping of the cell surface that is based on a scanned nanopipette. This allows high resolution, non-contact imaging of the soft and responsive cell surface using the ion current that flows between an electrode in the nanopipette and bath for distance feedback control.[3] Recently we have made a major advance in the resolution of the topographic images, by scanning with fine quartz pipettes, so we can directly visualise protein complexes on the surface of live cells.[4] The pipette can also be use to perform local nanoscale assays on the cell surface so as to perform single channel recording[5] or apply pressure to probe the mechanical properties. We have also combined high resolution topographic imaging with simultaneous recording of the fluorescence from the cell surface.[6] In addition the pipette can be used for controlled voltage driven delivery and deposition of biomolecules down to the single molecule level[3] and this is being used to probe the structure of the cell membrane using single molecule fluorescence tracking.

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[6]. PNAS 99 , 16018-16023 (2002)

8:40am **BI+NC-WeM3 Theory of Single Molecule Characterization using Random Telegraph Signals**, ***S. Vasudevan***, *K. Walczak, A.W. Ghosh*, University of Virginia

The future of nanoelectronics will depend not only on the capability to engineer 'smart' materials, but also on the ability to exploit new quantum phenomena that emerge at submicroscopic length scales. Molecular electronics has often been advocated as an ideal successor to silicon-based, complementary metal oxide semiconductor technology (CMOS). But its development has been thwarted by problems like poor gatability and low mobilities. Therefore we need to explore hybrid devices that do not compete with CMOS, but instead add novel functionalities by exploiting properties that are unique to molecules, such as their tendency to function as strongly correlated systems. Thus we need to account for more complex effects than usual semiclassical theory provides. In this paper we develop a theory for a new class of electronic devices that exploit correlated quantum scattering in a transistor channel. In these devices, molecules attach to the surface of a transistor channel; the chemical bonding leads to the transfer of charge and spectral weight between the molecule and the silicon surface. The overlap of molecular and silicon wavefunctions serves to passivate existing surface states as well as to create new localized molecular trap levels inside the silicon band-gap. At resonance driven by a gate, the traps are stochastically filled and emptied by the channel electrons, blocking and unblocking the channel. The resulting two-state random telegraph signal (RTS) can be used to locate the trap position both spectrally as well as spatially. This allows us to characterize and detect molecular species through unique nano-'barcodes'. The effect is enhanced in modern nanodevices as they can be fabricated practically defect free with near ballistic levels of operation. In contrast with ChemFETs, where one detects a single threshold shift for a specific molecule, here we get an entire spectral nano-'barcode' that can be compared against a compilation of theoretical responses to characterize and sense a molecular species. Since these devices operate by modulating surface properties of transistors, we call them 'SurfFETs'. The significant advantage of such SurfFETs is their exclusive detection of only molecules that overlap significantly with the channel to cause a transfer of states. This means that this electronic detection scheme is selective and inherently avoids false positives- clearly an advantageous feature for detection of molecules.

9:00am **BI+NC-WeM4 Magnetic Tweezers Measurement of the Bond Lifetime-Force Behavior of the IgG-Protein A Specific Molecular Interaction**, *H. Shang,* MagSense Life Sciences, ***G. Lee***, University College Dublin, Ireland

The bond lifetime-force behavior of the immunoglobulin G (IgG)-protein A interaction has been studied with magnetic tweezers to characterize the physical properties of the bond under nonequilibrium conditions. Superparamagnetic microparticles were developed that have a high and uniform magnetization to simultaneously apply a piconewton scale tensile force to many thousands of IgG-protein A bonds. A strong and a weak slip bond were detected with an effective bond length that is characteristic of short-range, stiff intermolecular interactions. These bonds are attributed to the interaction of protein A with the constant region (Fc) and heavy chain variable domain (VH) of IgG, respectively. The IgG-VH interaction appears to be one of the weakest specific molecular interactions that has been identified with a single molecule force measurement technique. This study demonstrates that magnetic tweezers can be used to rapidly characterize very weak biomolecular interactions as well as strong biomolecular interactions with a high degree of accuracy.

9:20am **BI+NC-WeM5 Elasticity Mapping of Pore Suspending Native Cell Membranes**, ***A. Janshoff***, Institute of Physical Chemistry, Germany

The mechanics of cellular membranes is governed by a non-equilibrium composite framework composed of semiflexible filamentous cytoskeleton and extracellular matrix proteins linked to a lipid bilayer. Non-local elasticity information of native cell membranes has so far been gathered by micropipette suction and rheological whole cell experiments. Locally confined measurements were conducted by using membrane-attached beads pulled by laser tweezers and by atomic force microscopy of entire cells. As yet, local mechanical information (elasticity maps) of isolated cellular membranes, such as basolateral membranes of endo- and epithelial cells, are however, not available. Here, we introduce a novel approach that allows the mapping of mechanical properties of native freestanding cellular membranes on a nanometer length scale. Basolateral membranes of polar epithelial MDCK II cells, prepared on a highly ordered porous substrate, were locally indented with the aim to unravel how the cytoskeleton and extracellular matrix (ECM) affects the viscoelasticity of such native membranes on a predefined length scale. We found a strong relation between the density and cross-linking of actin filaments and membrane stiffness.

9:40am **BI+NC-WeM6 Size Measurement of Targeted Nanoparticle Delivery Systems**, ***N. Farkas***, *J.A. Dagata, V.A. Hackley,* National Institute of Standards and Technology, *K.F. Pirollo, E.H. Chang*, Georgetown University Medical Center

The mean size and size distribution of a targeted nanoparticle delivery system (NDS) strongly influences the intrinsic stability and functionality of this molecular complex, affects its performance as a systemic drug delivery platform, and ultimately determines its efficacy towards early detection and treatment of cancer. Since its components undergo significant reorganization during multiple stages of self-assembly, it is essential to monitor size and stability of the complex throughout NDS formulation. Furthermore, reproducible and quantitative size measurement of individual entities, not only average properties of the entire population, is needed to assure potency and manufacturability of a specific formulation prior to entering clinical trials. Scanning probe microscopy (SPM) is capable of providing both high-resolution imaging of intact NDS immobilized on a substrate under fluid conditions and statistically meaningful, number-weighted averaged data for the complex. This presentation describes robust sample preparation methods and statistical image analysis of targeted liposome-based NDS with encapsulated therapeutic and diagnostic agents.[1] We present detailed examples of how variations in NDS formulation impact the size and stability of complexes with various payloads. These measurements are then compared with mean particle size distributions obtained by dynamic light scattering (DLS). SPM-based size distribution measurement technique in combination with DLS offers quantitative means of assessing size and stability, optimizing of formulation during drug development, and quality control during manufacturing of NDS.

[1] J. A. Dagata, N. Farkas, C. L. Dennis, R. D. Shull, V. A. Hackley, C. Yang, K. F. Pirollo, and E. H. Chang, Physical characterization methods for iron-oxide contrast agents encapsulated within a targeted liposome-based delivery system, Nanotechnology, in press.

10:40am **BI+NC-WeM9 Biophotonics: Resonant Detection of Single Molecules**, ***A.M. Armani***, University of Southern California **INVITED**

For many biological and chemical experiments, a sensor must have high sensitivity, high specificity, and fast response time. There are many technologies which are able to achieve one or two of these three requirements, but many still face fundamental sensitivity or response limitations. Silica optical resonators are able to overcome these limitations because of the high quality factor (Q).[1],[2] In their application as a single molecule sensor, the sensitivity is derived from the long photon lifetime inside the microcavity, and the specificity is derived from functionalization of the silica surface. During the initial series of label-free detection experiments, pure Interleukin-2 (IL-2) solutions were injected into the volume surrounding the microtoroid. The microtoroid successfully detected step-like shifts in resonance wavelength from individual IL-2 molecules binding. Additional experiments have shown that even in the more complex environment of serum individual binding events of IL-2 are still resolved.[3] Therefore, this single molecule sensor will enable research in new areas of biophysics and cell biology. Acknowledgements: The author would like to thank Prof. Richard Flagan, Prof. Scott Fraser, and Dr. Rajan Kulkarni at the California Institute of Technology. A.M. Armani is supported by the Provost's Initiative for Biomedical Nanoscience and the WiSE Program at the University of Southern California.

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11:20am **BI+NC-WeM11 Single-Molecule Detection and Mismatch Discrimination of Unlabeled DNA Targets**, ***M. Gunnarsson***, Chalmers Institute of Technology, Sweden, *P. Jönsson, J. Tegenfeldt,* Lund University, Sweden, *F. Höök*, Chalmers University of Technology, Sweden

Ultrasensitive biological sensors for low-abundant DNA and protein detection have emerged as an important tool for improving biomedical diagnostics, drug discovery, forensic analysis, but also advanced bioanalytical assays in fundamental research. We report on a single-molecule readout scheme based on total internal reflection fluorescence microscopy (TIRFM) demonstrating a detection limit in the low fM regime for short (30 mer) unlabeled DNA strands. Detection of DNA targets is accomplished by mediating the binding of suspended fluorescently labeled DNA-modified small unilamellar vesicles (~100 nm in diameter) to a DNA-modified substrate by unlabeled complementary single-stranded DNA. On top of rapid and sensitive detection, the technique is also shown capable of extracting kinetic data from statistics of the residence time of the binding reaction in equilibrium, i.e. without following neither the rate of binding upon injection nor release upon rinsing. The potential of this feature is demonstrated by discriminating a single mismatch from a fully complementary 30-mer DNA target.[1] The proposed detection scheme is particularly appealing due to the simplicity of the sensor, which relies on self-assembly principles and conventional TIRFM. In contrast to most other single-molecule detection schemes the imaging mode also offers possibilities for multiple spots to be measured simultaneously in an array-based design. The proposed sensor holds particular promise in cases when information about binding kinetics is valuable, such as in single nucleotide polymorphism (SNP) diagnostics.

[1] Gunnarsson, A., et al., Single-molecule detection and mismatch discrimination of unlabeled DNA targets. Nano Letters. 8(1): p. 183-188, 2008.

11:40am **BI+NC-WeM12 Optical Fiber Microarrays for Single Molecule Detection**, ***H.H. Gorris***, *D.R. Walt*, Tufts University

Optical fiber microarrays have been employed for the detection of single enzyme molecules. Single enzyme molecules were enclosed with fluorogenic substrate in an array of 50,000 individually addressable microchambers etched into a glass optical fiber bundle. The large array size provided excellent statistics. The substrate turnover in the microchambers was monitored with epifluorescence microscopy. We have observed a broad distribution of discrete turnover rates of single ß-galactosidase molecules that can be attributed to different enzyme conformations. When a slow-binding inhibitor was added to single ß-galactosidase molecules inhibited and active states of ß-galactosidase could be clearly distinguished. With a pre-steady-state experiment, we demonstrated the stochastic character of inhibitor release, which obeys first-order kinetics. Under steady-state conditions, the quantitative detection of substrate turnover changes over long time periods revealed repeated inhibitor binding and release events, which are accompanied by conformational changes of the enzyme's catalytic site. We proved that the rate constants of inhibitor release and binding derived from stochastic changes in the substrate turnover are consistent with bulk-reaction kinetics. Furthermore, we have applied the optical fiber microarray to the detection of single horseradish peroxidase molecules. These monomeric enzyme molecules exhibit a narrower distribution of turnover rates than the tetrameric ß-galactosidase, which could be explained by the number of catalytic sites involved in substrate turnover.

Biological, Organic, and Soft Materials Focus Topic
Room: 201 - Session BO+AS+BI+NC-WeM

Organized and Structured Organic Interfaces
Moderator: J.S. Shumaker-Parry, University of Utah

8:00am **BO+AS+BI+NC-WeM1 New Approaches to Chemical Lithography on the Micro- and Nanometer Length Scales**, ***N. Ballav***, *S. Schilp,* Universität Heidelberg, Germany, *T. Winkler, H. Thomas, A. Terfort,* Philipps-Universität Marburg, Germany, *M. Zharnikov*, Universität Heidelberg, Germany

The development of novel approaches for the fabrication of nanostructures and, in particular, chemical and biological patterns is an important technological and scientific challenge. One of the perspective methods applies a modification of chemisorbed monomolecular films - self-assembled monolayers (SAMs), which are well-ordered 2D-assembles of long-chain molecules attached to a suitable substrate. A flexible molecular architecture of the SAM constituents allows us to use a wide range of substrates, whereas the molecular size of these constituents makes SAMs an ideal platform for the fabrication of micro- and nanostructures. We present here two new approaches for the fabrication of chemical patterns with aliphatic SAMs as templates. Both approaches rely upon electron beam or X-ray lithography, but require much lower patterning dose as compared to already available methods as, e.g., Chemical Lithography with aromatic templates. The first technique is based on irradiation-promoted exchange reaction (IPER) between the primary SAM template and potential molecular substituent and can utilize a broad variety of commercially available molecules. The key idea of the second method is irradiation-induced activation of amino tail groups of the primary amino-terminated SAM template. Feasibility of both techniques is demonstrated by the fabrication of complex polymer micro- and nanobrushes in a broad height range and, in the case of the IPER approach, by the preparation of micron-scale gradients of protein adhesion.

8:20am **BO+AS+BI+NC-WeM2 Reversible Activation of a Polyelectrolyte Brush: Responsive Monolayers**, ***R. Steitz***, Hahn-Meitner-Institut Berlin, Germany, *V. Papaefthimiou,* TU Berlin, Germany, *J.U. Günther, C.A. Helm,* University of Greifswald, Germany, *S. Förster,* University of Hamburg, Germany, *G.H. Findenegg*, TU Berlin, Germany

Polyelectrolytes anchored on surfaces are important in various applications and are also a challenging topic for fundamental studies. In this work, a monolayer of the PEE114-b-PSS83 [(poly(ethyl ethylene)114-b-poly(styrene sulfonic acid)83] diblock copolymer was transferred from the air/water interface to a deuterated polystyrene coated silicon (dPS/Si) surface, for evaluation as a tunable polyelectrolyte brush containing system. The grafting density of the polymer film was controlled by changing the lateral pressure during the depositions. X-ray Reflectivity and AFM measurements showed that a homogeneous layer of the block copolymer was formed, whose thickness (maximum 8 nm) increased with increasing grafting density. Neutron reflectivity studies against aqueous solutions revealed a hydrophobic PEE layer attached on the dPS/Si surface, and a carpet/brush polystyrene sulfonate (PSS) double layer in water. The effect of salt concentration on the brush nanostructure was investigated in aqueous solutions containing 0-1 M NaCl. It was found that the brush thickness decreases for salt concentrations above 0.1 M. In addition, reversible activation of the brush by changing the ionic strength of the subphase was demonstrated. These results confirm a potential use as a stimuli-responsive polymer for both fundamental studies and biological applications.

9:20am **BO+AS+BI+NC-WeM5 Phase Stability of 2D and 3D Structures of Oligopyridines on HOPG Studied by Thermal Desorption Spectroscopy and Scanning Tunnelling Microscopy**, ***M. Roos***, *H.E. Hoster, R.J. Behm*, Ulm University, Germany

Bis(terpyridine)derivatives (BTP) form highly ordered hydrogen bonded 2D networks on graphite (HOPG) at both the solid/liquid[1,2] and the solid/gas[3,4] interface. As found by STM at both interfaces, these adlayer structures depend on the positions of the N-atoms within the molecules, which can be varied via the synthesis process.[1,2] Complementary to the STM studies, we have performed temperature programmed desorption experiments of two

different types of BTP molecules on HOPG. This revealed quantitative insights into phase stabilities of 2D (monolayer) and 3D (multilayer) phases. As to be expected from their large mass (618 amu) and correspondingly large moment of inertia, the translational and rotational degrees of freedom are found to play an important, even dominant role for the stability of more or less densely packed phases. This becomes apparent in strongly differing pre-exponential factors for desorption out of 2D and 3D phases. In agreement with STM observations at 300 K, the most stable phase (i.e., the one with the lowest chemical potential) for both molecules is not a close packed hydrogen bonded one, but a dilute 2D gas with facilitated translation and planar rotation. In this picture, ordered, hydrogen bonded structures observed at room temperature are only stabilized by the strong molecule-substrate interaction that allows enforcing higher coverages that go along with the more densely packed, ordered structures.

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9:40am **BO+AS+BI+NC-WeM6 Dielectric Spectroscopy for Biological Applications**, ***C. Prodan***, *C. Bot*, New Jersey Institute of Technology

Dielectric spectroscopy (DS) is a widely used technique to study the properties of cells, proteins and DNA in a fast, label free and noninvasive way. It measures the complex dielectric permitivities as a function of frequency for the given sample. Cellular membrane potential is on of the most important parameter of a living cell and represents the voltage difference between the inside and outside of a cell. Usual values of the membrane potential are in the range of 100 mV. Across a membrane of 2nm thick, this means electric fields of half million V/cm. Theoretical studies have shown that the membrane potential plays a dominant role on the dielectric permitivity of a cell suspension at low frequencies (0Hz-1kHz). Thus the membrane potential can be obtained from a simple measurement of the cell suspension dispersion curves. This talk presents the application of DS to measure and monitor the membrane potential from the low frequency dispersion curves of living cell suspensions of bacteria and mammalian cells. This technique is tested against the standard techniques for measuring the membrane potential such as patch clamping or voltage sensitive dyes.

10:40am **BO+AS+BI+NC-WeM9 True All-Organic Epitaxy in Fashionable Organic Hetero-Junctions**, ***G. Bussetti***, *C. Goletti, P. Chiaradia,* Università degli Studi di Roma Tor Vergata, Italy, *M. Campione, L. Raimondo, A. Sassella, A. Borghesi*, Università Milano-Bicocca, Italy **INVITED**

The improvement of charge transport performances and the control of related electronic properties (a crucial step in the development of organic electronic devices) are strictly connected to the quality of the organic-organic interface, that up to now has been limited by three main problems: 1) the molecular package in organic crystals is significantly influenced by the sample size. It is a difficult task to grow a large single crystal: the substrates commonly used are often assemblies of smaller crystals with different orientations; 2) the chemical and physical properties of the substrate surface play a key role during the build-up of the organic layer. When an organic crystal is growing, significant changes (due to desorption, molecular readjustment, roughness variation, layer erosion, etc.) occur in the freshly deposited surface. Unfortunately, in-situ and real time spectroscopies are not yet commonly applied to monitor this complex phenomenology; 3) an effective thermodynamic strategy during the arrangement of the organic etero-junction -as in inorganic Molecular Beam Epitaxy- (e.g., control of substrate temperature and sample growth rate, choice of single or multi-bunch growth, etc.) is still lacking in the deposition process. As a matter of fact, only recently a true all-organic epitaxy has been achieved. In this talk, the successful work and the most representative results we obtained in the last five years will be presented, showing that concrete possible solutions to the above mentioned points have been found. In particular, we have succeeded in growing different single organic crystals, namely α-quaterthiophene (α-4T), α-sexythiophene (α-6T), tetracene, rubrene, etc., with different shape, size (up to several square mm's) and orientation. An accurate investigation of the morphological and optical properties of the bare substrate as well as of the freshly grown ultra-thin organic layers has been performed. Our results demonstrate that the organic layer exhibits a high sensitivity to very low amount of contaminants. Moreover, we will show that is possible to tune the crystal growth from a Stransky-Krastanov to a Frank-van der Merve mode during the layer deposition of different organic compounds [α-4T, α-6T, tetracene, rubrene, etc.] by Organic Molecular Beam Epitaxy. In conclusion, the growth of various organic heterojunctions with epitaxial quality is now a gain result.

11:20am **BO+AS+BI+NC-WeM11 Modification of Self-Assembled Monolayer Surfaces Using Hyperthemal Ion Beams**, ***J. Laskin***, *P. Wang, O. Hadjar*, Pacific Northwest National Laboratory **INVITED**

Collisions of ions with surfaces play an important role in a variety of scientific disciplines including surface science, materials science, mass spectrometry, imaging and spectroscopy. This presentation will focus on phenomena that occur during collisions of complex polyatomic ions with surfaces at hyperthermal energies with specific emphasis on ion deposition, charge transfer, bond making and bond breaking processes. Because in this energy regime the initial kinetic energy of the ion is commonly sufficient for breaking chemical bonds on the surface but is too low for significant penetration of a polyatomic ion into the surface, hyperthermal collisions are well suited for chemical modification of the outer layer of the surface without substantial perturbation of the bulk substrate. In addition, careful control of the properties of the ion beam can be used for very specific surface modification. Covalent and non–covalent immobilization of peptides using soft-landing of mass-selected ions onto inert and reactive self-assembled monolayer surfaces will be discussed. Fundamental principles derived from such studies are relevant to the understanding of the transport of biomolecules through membranes in living organisms and provides a clear pathway for highly-selective preparation of biological surfaces.

Biomaterial Interfaces
Room: 202 - Session BI-WeA

Quantitative Analysis of Biointerfaces
Moderator: D.G. Castner, University of Washington NESAC/BIO

1:40pm **BI-WeA1 'Structure' of Water: Myth or Reality?**, ***M. Grunze***, University of Heidelberg, Germany **INVITED**

The "structure" of water at interfaces is a reoccurring interpretation of experimental data or computer simulations employing simplified models for liquid water. Oriented binding of water molecules to a hydrogen bridge bond donor or acceptor group, as observed e.g. in vibrational spectroscopies, does not imply translational symmetry or "structure", and the orientational order parameter decays typically with the second hydration shell. Whereas the practical significance of surface energy and hence wetability of organic surfaces for adhesion, adhesion failure and biomolecule interaction is obvious, the molecular basis of the hydrophobic and hydrophilic properties of organic surfaces is poorly understood. This is due to the lack of suitable experimental tools for detailed spectroscopy studies of organic interfaces in liquid environments and of molecular detailed theoretical descriptions of hydrophobic/hydrophilic interactions. In this talk I will briefly discuss the limits and potentials of experimental approaches to interfacial water (Neutron Reflectivity, Sum Frequency Generation Spectroscopy, and Terahertz Spectroscopy) to develop a molecular understanding of the properties of water in organic interphases of different surface energy; and time permitting get back to a long standing controversy, the "inert" surface problem, which is a good example for demonstrating the complexity of interfacial water properties.

2:20pm **BI-WeA3 A Novel Approach to XPS Characterisation of 'Click' Surface Chemistry, More Information Less Damage**, ***T.S. Nunney***, *R.G. White*, Thermo Fisher Scientific, UK, *N.B. Larsen, T.S. Hansen, A.E. Daugaard, S. Hvilsted*, Technical University of Denmark

'Click' chemistry is increasingly used for chemical surface engineering of polymer devices to be used in biological and medical applications. Advantages of 'click' chemistry include mild reaction conditions, i.e. aqueous environment at room temperature, and high chemical specificity of the coupling. We recently demonstrated surface engineering of ultrathin electrically conductive polymer films by 'clicking' organic functional units that control wettability, protein adhesion, or fluorescence, all functions of major relevance to biomedical applications. The most commonly used click reaction is based on the coupling of organic azides to alkynes. This is also the basis of our recently reported functional monomer, azide modified 3,4-ethylenedioxythiophene, for conducting polymer films (PEDOT-N3) reactive towards alkyne functionalized molecular species. The ability of X-ray photoelectron spectroscopy (XPS) to provide quantitative chemical state information makes it ideal for the investigation of the resultant clicked surface chemistry. In the example above, differences in the XPS binding energy for the azide and triazole nitrogens serve as a useful method to determine if the click reaction has completed successfully. It is known, however, that degradation of the azide chemistry during XPS measurement process can significantly obscure the result. In this paper we will discuss methods for minimisation of the measurement-induced chemical degradation. These methods rely on a number of hardware and software features which have recently become available on modern XPS instrumentation. The methods described require the layers to be uniform so that the data can be collected as a map, thus reducing the X-ray and electron flux density during the measurement. Deconvolution routines will be shown to facilitate the rapid chemical state mapping of patterned variants of these surfaces.

2:40pm **BI-WeA4 A New QCM-D and Reflectometry Instrument - Applications to Supported Lipid Structures and their Interactions**, ***M. Edvardsson***, *S. Svedhem*, Chalmers University of Technology, Sweden, *G. Wang*, Chalmers University of Technology and Q-Sense AB, Sweden, *R. Richter*, CIC biomaGUNE, Spain, *M. Rodahl*, Q-Sense AB, Sweden, *B. Kasemo*, Chalmers University of Technology, Sweden

In the past decade, the Quartz Crystal Microbalance with Dissipation monitoring technique (QCM-D) has emerged as a powerful biosensor technique.[1] A key feature of the technique is that the shift of the resonant frequency, Δf, obtained upon adsorption of mass on the QCM-D sensor surface includes both the actual mass and solvent (e.g. water) associated with it. For a rigid film containing no water (low dissipation shifts, ΔD), the frequency shift, Δf, can be considered proportional to the mass of the film. For viscoelastic films containing water (high dissipation shifts), however, it is difficult to determine how much of the frequency shift results from the actual adsorbed mass and how much is a contribution from entrapped or associated water. In some applications, the signal enhancement that is obtained through the associated liquid, makes the QCM-D technique unique with respect to the added information that is gained compared to, for example, optical techniques. In particular, spontaneous fusion of lipid vesicles onto solid supports have been studied extensively using the QCM-D technique,[2] and unique new information has been obtained. However, for a full picture one would, for such complex viscoelastic films, ideally combine the QCM-D technique with a technique that allows separation of the adsorbed (non-hydrated or "dry") mass and the associated liquid (wet mass). This presentation demonstrates applications of a recently developed instrument, combining, on the same sensor surface, the QCM-D technique and optical reflectometry [Wang et al., submitted to Rev. Sci Instr,], for surface based analysis of biomolecular and polymer adlayers. The combination instrument makes it possible to do simultaneous, time-resolved measurements of hydrated and non-hydrated mass and viscoelastic properties of films and molecular adlayers formed on the surface. The experimental setup is described, and the value of this combination of techniques is demonstrated via applications on model systems that involve supported lipid structures of various degree of hydration; ranging from systems of low water content, e.g., bilayers, to those of high water content, such as surface-attached vesicles and bilayers with a highly hydrated peptide coupled to it.

[1]Cooper, M. A.; Singleton, V. T. J. Mol. Rec. 2007, 20, 154-184.
[2]Richter, R. P.; Bérat, R.; Brisson, A. Langmuir 2006, 22, 3497-3505.

3:00pm **BI-WeA5 Calculation of Adsorption Free Energy for Peptide-Surface Interactions using Molecular Dynamics Simulation Methods**, ***N. Vellore***, *S.J. Stuart*, Clemson University, *B.R. Brooks*, National Institutes of Health, *R.A. Latour*, Clemson University

While it is well understood that protein-surface interactions are of fundamental importance for understanding cell-surface interactions, very little is understood at this time regarding the molecular level events that control protein adsorption behavior. Molecular dynamics simulations methods have enormous potential for development as a tool to help understand and predict protein adsorption behavior. These methods, however, must first be developed and validated for this specific application. One of the most important areas for development is the assessment and validation of force field parameters that will enable the competition between amino acid residues of a peptide or protein and molecules of the solvent (i.e, water and salt ions) for the functional groups presented by a surface. One of the fundamental driving forces that control these types of interactions is the free energy of adsorption. We have therefore developed a method of accurately calculating the adsorption free energy of peptide-surface interactions using molecular dynamics simulations with an advanced sampling algorithm called biased replica-exchange molecular dynamics (biased-REMD). Simulations are performed with the CHARMM force field and simulation package using explicitly represented solvent (150 mM Na+/Cl- in TIP3P water) with periodic boundary conditions. A host-guest peptide model is used for these simulations in the form of TGTG-X-GTGT, where the T (threonine) and G (glycine) flanking sequences are the host residues and X represents a variable guest residue. Alkanethiol self-assembled monolayers (SAMs) with a broad range of polymer-like functionalities are being used as the adsorbent surfaces. The results of these simulations are being compared with complementary experimental studies using these same peptide-SAM systems in order to evaluate the accuracy of the force field, and to provide a basis for force field parameter modification for the development of a validated force field parameter set for the accurate representation of peptide-surface interactions. Once developed, these methods will be able to be applied to accurately simulate protein-surface interactions, thus providing a valuable resource to investigate protein-surface interactions at the molecular level.

4:00pm **BI-WeA8 Nonlinear Light Scattering: Bridging the Gap between Surface Science & Soft Matter**, ***S. Roke***, Max-Planck Institute for Metals Research, Germany **INVITED**

Interfaces play a key role in many processes. They play a regulating role in transport and structural phenomena in biological cells, they can determine the chemistry and (phase) behavior of colloidal systems, they are important for the mechanical properties of (amorphous) solids and they determine the electrical properties of micro- and nano-electronics. When the size of materials decreases down to the level of micro- or nano-structures, the relative interfacial area increases. For small systems it is well-known that the interfacial region becomes a dominating factor in determining the physical and chemical properties of a material. Thermodynamically, on a

macroscopic level, the effect of an interface region is well understood. On a molecular level, however, it is not. In this presentation I will introduce vibrational sum frequency scattering as a novel method to investigate particle and domain interfaces,[1] highlight new possibilities that become available and show some of the latest developments. These include: the possibility of investigating molecular surface effects in colloidal phase transitions,[2,3] how to extract molecular properties[4] and the possibility of observing embedded domain structures in polymorph materials.[5]

[1] - S. Roke, W. G. Roeterdink, J. E. G. J. Wijnhoven, A. V. Petukhov, A. W. Kleyn and M. Bonn, Phys. Rev. Lett., 91 (2003), 258302-1.

[2] - S. Roke, J. Buitenhuis, M. Bonn and A. Van Blaaderen, J. Phys.: Condens. Matter., 17 (2005), S3469-S3475.

[3] - S. Roke, J. Buitenhuis, A. van Blaaderen and M. Bonn, Proc. Nat. Acad. Sci, 103 (2006), 13310-13314.

[4] - A. G. F. de Beer and S. Roke, Phys. Rev. B, 75 (2007), 245438-1-8.

[5] - A. G. F. de Beer, H. B. de Aguiar, J. F. W. Nijsen, A. B. Sugiharto and S. Roke, submitted.

4:40pm **BI-WeA10 Characterization of DNA Monolayers on Gold using Sum Frequency Generation Spectroscopy**, ***C.L. Howell***, *M. Grunze, P. Koelsch*, University of Heidelberg, Germany

We investigated a series of model monolayers of single stranded DNA (ssDNA) on gold using broadband femtosecond Sum Frequency Generation (SFG) Spectroscopy. SFG processes, involving a non-linear resonant responses produced by exciting vibrational modes of molecular bonds using overlapping IR and visible beams, are inherently interface-specific. The surface specificity of SFG, combined with polarization dependence, allows for the investigation of the ordering and orientation of molecules at surfaces in air and through bulk solutions. However, due to the difficulties associated with operating an SFG system in biological relevant spectral regions (such as the amide I and fingerprint), combined with the difficulties of interpreting vibrational spectra from complex biomacromolecules, there are few published examples of characterization of DNA films using SFG. Our goal is to create high quality vibrational SFG spectra of model monolayers of DNA on gold and to compare these spectra to results obtained from complementary surface spectroscopies that have been applied to DNA monolayers, such as XPS, FTIR, and NEXAFS. Preliminary results in the C-H stretching region show distinctive changes in the locations and relative intensities of peaks for a film of thiol-modified 5-mers of thymine (T5-SH) compared to thiol modified 25-mers (T25-SH) and unmodified thymine 5-mers (T5). Preliminary SFG spectra of ssDNA films in the Amide I region revealed changes in the locations and intensities of the major peaks for the T5 film compared to a film of unmodified adenine 5-mers, as well as compared to T5-SH and T25-SH films. Refinement and validation of SFG Spectroscopy as a tool for the characterization of DNA monolayers on gold could provide another method for examining the structure of these films, and potentially serve as a bridge for comparing these systems ex situ and in situ.

5:00pm **BI-WeA11 Avoiding Parasitic Reactions Due to Interconnect Dead Volume and Non-Specific Binding in Microfluidics**, *X. Luo,* ***D.L. Berlin***, *W.E. Bentley, G.F. Payne, R. Ghodssi, G.W. Rubloff*, University of Maryland

Biological microelectromechanical systems (bioMEMS) provide an attractive approach to understanding and modifying enzymatic pathways by separating and interrogating individual reaction steps at localized sites in a microfluidic network. We have previously shown that electrodeposited chitosan enables immobilization of an enzyme at a specific site while maintaining its catalytic activity. While promising as a methodology to replicate metabolic pathways and search for inhibitors as drug candidates, these investigations also revealed unintended (or parasitic) effects, including products generated by the enzyme either (1) in the homogeneous phase (in the liquid), or (2) nonspecifically bound to microchannel surfaces. Here we report on bioMEMS designs that significantly suppress these parasitic effects. To reduce homogeneous reactions we have developed a new packaging and assembly strategy that eliminates fluid reservoirs that are commonly used for fluidic interconnects with external tubing. To suppress reactions by nonspecifically bound enzyme on microchannel walls we have implemented a cross-flow microfluidic network design so that enzyme flow for assembly and substrate/product flow for reaction share only the region where the enzyme is immobilized at the intended reaction site. Our results show that the signal-to-background ratio of sequential enzymatic reactions increases from 0.72 to 1.28 by eliminating the packaging reservoirs, and increases to 2.43 by separating the flow direction of enzymatic reaction from that of enzyme assembly. These techniques can be easily applied to versatile microfluidic devices to minimize parasitic reactions in sequential biochemical reactions.

5:20pm **BI-WeA12 Probing Orientation and Conformation of α-Helix and β-Sheet Model Peptides on Self-Assembled Monolayers with SFG and NEXAFS Spectroscopy**, ***T. Weidner***, *J. Apte, L.J. Gamble, D.G. Castner*, University of Washington

Understanding the interaction of proteins and peptides with engineered surfaces from first principles is essential for the design of biomaterials which are applicable in antifouling, implant technology and immunosensors. Controlled immobilization of peptides onto artificial biointerfaces plays a key role in these technologies and it is of crucial importance to develop tools to examine interfacial properties of adsorbed peptides such as orientation, and secondary structure. In this study we used sum frequency generation (SFG) vibrational spectroscopy and near edge X-ray absorption fine structure (NEXAFS) spectroscopy to characterize the structure of α-helix and β-strand model peptides on self-assembled monolayers (SAMs). The formation of peptide monolayers was confirmed using X-ray photoelectron spectroscopy. The α-helix peptide is a 14-mer and the β-strand is a 15-mer of hydrophilic lysine (K) and hydrophobic leucine (L) residues with a hydrophobic periodicity of 3.5 and 2, respectively. Both peptides have the hydrophobic side-chains on one side and the hydrophilic on the other. The SAMs used as hydrophobic and hydrophilic model surfaces were prepared from alkane thiols on gold having either charged COOH or hydrophobic CH_3 units as terminal groups. For SFG studies we used the deuterated analog of the latter SAM. SFG spectra collected in the C-H region exhibit strong peaks near 2965 cm^{-1}, 2940 cm^{-1} and 2875 cm^{-1} related to ordered leucine side chains on both surface chemistries. The relative phase of these features revealed the orientation of the leucine side chains. On COOH a relative phase of 1.4 and 1.6 rad for α-helix and β-strand, respectively, showed that the leucine was oriented away from the surface while a phase of 0 rad for both peptides on CH_3 proved that the leucines are oriented towards the interface. Amide I peaks observed at 1656 cm^{-1} for the α-helix peptide confirm that the secondary structure is preserved on both SAMs. A strong linear dichroism related to the amide π^* orbital at 400.1 eV was observed in the nitrogen K-edge NEXAFS spectra for the β-strand peptides on both surfaces, suggesting that the peptides are oriented parallel to the surface with the side-chains normal to the interface. For the α-helix the dichroism of the amide π^* is weak, probably due to the broad distribution of amide bond orientations for this secondary structure.

Biological, Organic, and Soft Materials Focus Topic
Room: 201 - Session BO+AS+BI-WeA

Advances in Surface Analytical Methods for Organic and Biological Interfaces

Moderator: D.W. Grainger, University of Utah

1:40pm **BO+AS+BI-WeA1 In-situ Broadband Sum-Frequency Spectroscopy of Biomolecules at Interfaces**, ***P. Koelsch***, *V. Kurz, R. Schmidt,* University Heidelberg, Germany, *C.L. Howell,* University of Maine, *M. Grunze*, University Heidelberg, Germany

Sum-frequency generation (SFG) spectroscopy has been employed to characterize a variety of biointerphases in extended spectral regions in aqueous environment. This contribution summarizes our recent SFG studies on DNA films, extracellular matrix compounds, switchable self assembled monolayers, and other biological molecules. Molecular level details revealed in these studies show that SFG offers the prospect of characterizing conformation, orientation and ordering of biological molecules at interfaces in situ. The technique of SFG spectroscopy is inherently surface specific having submonolayer resolution. Being an all-optical technique it can be operated in aqueous environment and even buried interfaces can be assessed. However, beside the obvious potential impact of SFG spectroscopy, to date, most studies of biological systems have only been performed in the CH and OH stretching vibration regions. This is related to the difficulties in generating tunable high energy light pulses with table top laser systems at the biologically relevant lower wavenumber region (amide and fingerprint) to gain SFG spectra with reasonable signal to noise ratios. In this contribution we show, that our broadband femtosecond SFG spectrometer provides spectral data in the amide I and fingerprint region in air and aqueous environment on a daily bases which opens the opportunity to characterize in situ orientation and conformation of a wider class of more complex biomolecules. We will summarize these biologically related SFG results and demonstrate the potential impact of this technique, also to introduce SFG spectroscopy as another method for examining biofilms ex situ and in situ.

2:00pm **BO+AS+BI-WeA2 Secondary Protein Structures in Barnacle Cement**, ***D.E. Barlow***, Nova Research, *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. While most work on the chemistry of barnacle adhesion to date has focused on identifying the protein composition of barnacle cement, relatively little has been done to directly characterize structure of barnacle cement proteins in their native states. Such studies should provide further insight into relationships between chemical structure and adhesion, as well as the types of biochemical mechanisms that may play roles in barnacle cement curing. We have used atomic force microscopy (AFM), circular dichroism (CD), and Fourier transform infrared (FTIR) spectroscopy to characterize cements deposited on quartz and CaF_2 substrates in seawater by barnacles (Amphibalanus amphitrite) transferred from silicone release panels. AFM imaging consistently shows that secondary cement residues left on the substrates are fibrillar. Circular dichroism spectra of cement residues on quartz showed negative peaks centered near 225 nm and positive peaks at about 195 nm, indicating that the barnacle cements are primarily helical in structure, but also contain some β-sheet components. This is further confirmed by transmission FTIR of cement residues on CaF_2, for which the amide III band is found to be composed of a broad band centered ~ 1650 cm^{-1} consistent with α-helical structures, and components near 1685 and 1630 cm^{-1} consistent with β-sheet structures. These results suggest that the fibrillar structures are predominantly helical in structure, in contrast with fibrillar structures like amyloids that exhibit primarily β-sheet conformations.

2:20pm **BO+AS+BI-WeA3 Analysis of Biosamples with Imaging TOF-SIMS**, ***H. Nygren***, *P. Malmberg*, University of Gothenburg, Sweden **INVITED**

Secondary ion mass spectrometry (SIMS) is based on the acceleration of primary ions onto a target. Secondary electrons, neutrals and ions are emitted from the target reflecting its chemical composition. SIMS is unique in its ability to detect simultaneously several target molecules in compound samples and to image their localization at subcellular resolution. The aim of the project is to develop the technology of imaging TOF-SIMS as a tool in biomedical research for analysis and localization of relevant target molecules e.g. inorganic ions, lipids, drugs, carbohydrates and proteins in the same sample. Recent research in TOF-SIMS has shown the distribution of a wide variety of compounds in biological tissue. The current issues in TOF-SIMS analysis are the data interpretation and identification of all new peaks detected with this technique. Until 2004, only a few high mass peaks of biological origin were identified, which leaves us in a situation where much of the interpretation of spectra remains to be done. The aim of this presentation is to define analyte molecules by mass spectra obtained using new primary ion sources with unique fragmentation properties. The possibility to detect proteins is of special interest. It is also an aim of the project to educate students in the analysis of cells and tissue with a high technology method as the research group is presently purchasing our own TOF-SIMS instrument.

3:00pm **BO+AS+BI-WeA5 A Laser Desorption Vacuum Ultraviolet Postionization Imaging Mass Spectrometer for Biological Sample Analysis**, *J.F. Moore,* MassThink LLC, *A. Akhmetov, G.L. Gasper,* University of Illinois at Chicago, *R. Carlson,* Montana State University, *M. Blaze,* ***L. Hanley***, University of Illinois at Chicago

Creating images with mass spectrometry (MS) at <10 micron scale resolution is a substantial challenge due to diffusion effects and low signal levels. A new instrument is described for laser desorption and in-source postionization (LDPI) imaging mass spectrometry with enhanced performance over the previous non-imaging instrument.[1] A significant fraction of the desorbed molecules are intercepted by a vacuum ultraviolet (VUV) postionizing laser, then extracted into the time-of-flight (TOF) mass analyzer. Postionization provides a higher and more consistent sensitivity than matrix-assisted laser desorption/ionization for certain analytes, thereby enhancing imaging MS on this small length scale. The instrument utilizes 349 nm laser desorption with 5 micron minimum spot size, 157 nm laser postionization and will operate at ~200 Hz. Samples are analyzed in microprobe mode and are imaged by rastering the x-y stage. The ion source also incorporates digital optical imaging of the sample surface, which allows for correlation of MS and optical images. The orthogonal geometry of the ion source provides for good separation of direct ion signal and postionized signal by varying the extraction delay, leading to enhanced mass resolution. Use of LDPI-MS is demonstrated for the detection of antibiotics within intact bacterial biofilms.[2] Staphylococcus epidermidis is a common Gram positive bacterium that resides on human skin and is one of the most frequent culprits behind hospital acquired biofilm infections. Treatment of biofilm infections is hindered by the limited ability of antibiotics to kill biofilm associated microbes. LDPI-MS is used to detect antibiotics within intact biofilms without significant interference from other biofilm chemical constituents. Sulfadiazine is detected these biofilms at relatively high concentrations while tetracylcine is detected at near clinical concentrations. These results indicate that imaging MS of bacterial biofilms, animal tissue, or other biological samples by LDPI-MS can be applied to analysis of low ionization potential analytes such as the described antibiotics and other compounds including pharmaceuticals.

[1]M. Zhou, C. Wu, A. Akhmetov, P.D. Edirisinghe, J.L. Drummond and L. Hanley, J. Amer. Soc. Mass Spectrom. 18, 1097 (2007).

[2]G.L. Gasper, R. Carlson, A. Akhmetov, J.F. Moore and L. Hanley, Proteom. (2008) in press.

4:00pm **BO+AS+BI-WeA8 3D Mass Spectrometric Analysis of Non-Dehydrated Biological Samples**, ***H.F. Arlinghaus***, *J. Möller, C. Kriegeskotte, D. Lipinsky*, Westfälische Wilhelms-Universität Münster, Germany

ToF-SIMS and laser-SNMS are increasingly important tools for analyzing the elemental and molecular distribution in biological samples. However, in-vivo analyses of tissues or cell cultures are impossible because the sample must accommodate the vacuum conditions of the instrument. Thus, fixing the sample in its vital state, such as freezing, is essential. Sample preparation by cryo-fractioning or cryosectioning techniques followed by freeze-drying has been successfully used. However, these techniques exhibit several limitations. In order to overcome these limitations, a combination of a ToF-SIMS/laser-SNMS instrument and an in-vacuum cryosectioning instrument were developed for directly preparing and analyzing frozen non-dehydrated samples. The correct sample temperature after preparation and during the analysis showed to be a major factor for the quality of suitable ToF-SIMS/Laser-SNMS analyses. On the one hand, it is necessary to keep the sample cold to avoid freeze drying, on the other hand, a slight increase of the sample temperature removes adsorbates formed by residual gas in the cutting chamber and, more importantly, water resulting from the cutting process, and anticipates adsorption during the analysis. In our presentation, we will show and discuss how to optimize the sample's temperature, sample preparation techniques for analyzing various biological samples, and the possibility of obtaining 3D molecular images of frozen non-dehydrated biological samples. Our data will show that both TOF-SIMS and Laser-SNMS are capable of imaging elements and molecules in complex biological samples and that they are very valuable tools in advancing applications in life sciences.

4:20pm **BO+AS+BI-WeA9 Construction of Complex Two- and Three-Dimensional Nanostructures: Combined Time-of-Flight Secondary Ion Mass Spectrometry and Microscopy Studies**, *C. Zhou, P. Lu,* ***A.V. Walker***, Washington University in St. Louis

Methods for the chemically selective deposition of metals, semiconductors, biomolecules and other compounds have been studied and applied to the construction of complex multilayer structures. This work has important applications in molecular and organic electronics, sensing, biotechnology and photonics. To illustrate our approach we present two examples: the chemical bath deposition (CBD) of zinc sulfide on functionalized SAMs and the construction of three-dimensional nanostructures via layer-by-layer growth. CBD is a solution-based method for the controlled deposition of semiconductors. ZnS is a direct band-gap semiconductor used electroluminescent devices and solar cells. Two sizes of crystallites are observed to form: ~500 nm nanoflowers and ~2 μm crystallites. Nanoflowers nucleate at Zn(II)-carboxylate terminal group complexes on -COOH terminated SAMs. They grow via an ion-by-ion reaction pathway and remain chemically bound to the SAM. In contrast, the micron-sized crystallites form in solution (cluster-by-cluster growth) and are observed on all SAMs studied (-OH, -COOH and $-CH_3$ terminated SAMs). These crystallites can be easily removed from the surface using sonication. Thus under the appropriate experimental conditions ZnS can be selectively deposited onto -COOH terminated SAMs. We illustrate this by selectively depositing ZnS on a patterned -COOH/$-CH_3$ terminated SAM surface. Our approach for the construction of three-dimensional nanostructures begins with a single SAM layer deposited and UV-photopatterned using standard techniques. A second layer is then assembled by specific chemical reaction with the terminal groups of the first SAM. Additional layers are deposited using the same method. Experiments to date have focused on the specific coupling reaction of amines with carboxylic acids to form multilayer structures. Using TOF SIMS imaging we have followed each step of this reaction and have been able to demonstrate the selective formation of multilayer structures on patterned -COOH/$-CH_3$ surfaces. This coupling is used to make several proof-of-concept multifunctional structures.

4:40pm **BO+AS+BI-WeA10 TOF-SIMS Analysis of Lipid Transfer between Vesicles and Supported Lipid Bilayers on TiO_2, *P. Sjovall,*** SP Technical Research Institute of Sweden, *A. Kunze, B. Kasemo, S. Svedhem*, Chalmers University of Technology, Sweden

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) was used to investigate the electrostatically driven lipid transfer between negatively charged vesicles (POPS) and a positively charged supported lipid bilayer (POEPC) on a TiO_2 surface. Quartz crystal microbalance with dissipation (QCM-D) was used to monitor the formation of the POEPC bilayer (by vesicle adsorption and rupture), the subsequent interaction with POPS vesicles, and the resulting lipid transfer between the bilayer and the vesicles. In addition, QCM-D showed that SDS treatment of the bilayer after lipid transfer removed mass corresponding to one of the lipid leaflets (seemingly leaving a lipid monolayer), and that a bilayer could be reformed upon POEPC vesicle adsorption on this monolayer. TOF-SIMS analysis using Bi_3^+ primary ions was used to provide quantitative estimates of the lipid composition in the different lipid layers. The lipid bilayers were prepared for TOF-SIMS analysis by plunge freezing and freeze drying(1). In order to allow for unambiguous detection of POPS in the lipid bilayers, POPS with fully deuterated palmitate fatty acid tail groups was used in the buffer vesicles. Quantitative estimates of the lipid compositions were made based on the signal intensities from the deuterated (POPS) and undeuterated (POEPC) palmitate ions, as well as from the oleate (POPS and POEPC) ion, using a simple model for the concentration-dependent TOF-SIMS signal intensities. Reference bilayers prepared with known lipid compositions were analysed in order to provide calibration points for the quantitative analysis. The results show that the POEPC bilayer after lipid transfer contains approximately 50% POPS, while the SDS-resistant monolayer contains about 70% POPS and the reformed bilayer contains 20-25% POPS. Possible asymmetries in the lipid composition of the bilayers were, however, not taken into account. A number of peaks, which can be assigned to molecular ions of POPS, were observed in the negative ion spectra of the POPS-containing lipid bilayers. Interestingly, these peaks were absent in the spectra from the SDS-resistant monolayer, although the signal from the deuterated palmitate ion indicated about 70% POPS in the monolayer. This suggests that the POPS molecular peak only shows appreciable intensity in TOF-SIMS spectra from the bilayer structure, similar to what has been observed previously for POPC.[1]

[1]Prinz et al., Langmuir 2007, 23, 8035-8041.

5:00pm **BO+AS+BI-WeA11 Spatial Distribution Analysis of a Selenium Based Anti-Cancer Drug in Tumor Tissue Samples by ToF-SIMS, *S.A. Burns,*** University at Buffalo, *M. Khin, L. Kazim, Y. Rustum, S. Cao, F. Durrani,* Roswell Park Cancer Institute, *J.A. Gardella*, University at Buffalo

Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) has recently found new applications in the field of tissue analysis due to the advancement of cluster ion sources.[1] Bi cluster primary ion sources have been shown to produce chemical images with high spatial resolution, to ca. 100nm. The use of C60 cluster ion sources for depth profile analysis distributes the ion impact force allowing for a more specialized analysis of organic samples.[2] ToF-SIMS is an extremely useful application to detect low molecular weight drugs within a polymeric membrane. This study utilized this application to spatially image drug distribution of an anticancer agent in a two types of tissue samples. Nude mice implanted with human head and neck tumors were treated with methylselenocystein (MSC), a known anticarcinogen.[3] MALDI imaging has been used to determine the distribution of another anti-cancer drug, CPT-11 when MSC has also been introduced.[4] The distribution of CPT-11 was found to be more even throughout the tumor in the presence of MSC. This indicates that the MSC causes an increase the vasculature of a tumor thereby allowing other anti-cancer drugs to distribute evenly. MALDI imaging was able to show drug distribution but could not associate the fragment peak of the MSC with the vasculature of the cells due to restrictions in image resolution (100μm). ToF-SIMS imaging has been shown to reach resolutions of 100nm allowing for this type of analysis to be performed. MSC treated tumor and liver samples were analyzed using imaging and depth profiling to determine the distribution of drug with respect to the vasculature of the tissue. The first step of this study was to determine characteristic peaks from the MSC that could be identified in the tissue samples. Analysis of the livers and tumors of the mice that had been treated with MSC had fragment peaks with isotopic distributions indicating selenium containing organic compounds not found in the control samples. These fragment peaks were used as the drug peaks whose distribution in the tissue samples were compared to fragment peaks which could be attributed directly to cell vascular structure.

[1]Brunelle,A,et al. Journal of Mass Spectrometry 2005,40,985-999
[2]Fletcher,JS,et al. Analytical Chemistry 2006,78,1827-31
[3]Azrak,RG,et al. Biochemical Pharmacology 2007,73,1280-1287
[4]Prieto Conaway,MC,et al. Thermo Scientific 2008,Application Note.

5:20pm **BO+AS+BI-WeA12 Effects of Different Sample Preparation Methods for Cell Imaging using TOF-SIMS, *J. Malm,*** SP Technical Research Institute of Sweden, *D. Giannaras,* University of Glasgow, UK, *P. Sjövall,* SP Technical Research Institute of Sweden, *N. Gadegaard, M.O. Riehle*, University of Glasgow, UK

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is increasingly being used for chemical imaging of cells and tissue. A concern in these studies is that the samples need to be prepared for the vacuum environment. Several sample preparation methods exist for this purpose. In this work, effects of different preparation methods on the structure and surface chemistry of human fibroblast hTert cells were studied. Two fixation protocols, using glutaraldehyde (GA, $C_5H_8O_2$), and osmium tetroxide (OsO_4), respectively, were compared to a non-fixing protocol where cells were washed with ammonium formate (AF, NH_4HCOO) prior to drying. Three drying techniques were compared, namely freeze-drying (FD) after rapid plunge-freezing, critical point-drying (CPD), and alcohol ladder-drying (ALD). Imaging TOF-SIMS with Bi_3 cluster primary ions was used to compare the different preparation protocols with respect to surface chemistry, and the structure of the cells after preparation was studied using scanning electron microscopy (SEM). For the AF-washed samples, changes in cell volume was followed by interference reflection microscopy (IRM). The results show that both the fixation/washing protocols as well as the drying protocols affect the chemical information obtained in TOF-SIMS analyses. For GA-fixed samples, both CPD and ALD give rise to reduced phosphocholine (PC) signal on the cell surface by two orders of magnitude, as compared to FD, while no significant differences are seen for cholesterol and amino acid fragment ions. GA-fixed samples post-fixed using OsO_4 showed PC intensities reduced by only one order of magnitude, going from FD to CPD or ALD. The cholesterol intensity was found to be higher for AF-washed cells and cells fixed with OsO_4, than for GA fixed cells. An increase in amino acid intensity going from AF to GA to OsO_4 was also observed.

Biomaterial Interfaces
Room: 202 - Session BI+NC-ThM

Engineering Biointerfaces
Moderator: S. Zauscher, Duke University

8:00am **BI+NC-ThM1 Engineering Membrane Physical Properties and Dynamics using Structured Interfaces**, ***A.N. Parikh***, *B. Sanii, A.M. Smith, M. Howland, A.M.A.M. Brozell*, University of California, Davis **INVITED**

Interfacial organization of lipids and amphiphiles into a discrete number of molecular layers provides,arguably, one of the most pristine experimental realizations of elf-organized, two-dimensional systems. it provides an experimental test-bed for the study of a rich variety of interface-dominated processes, including surface melting, low-dimensional phase transitions, surface dynamics, and phase coexistence and separation. This talk will present recent experimental evidence from our laboratories which highlight the importance of substrate structure (e.g., topography, charge, and surface energies) in engineering the physical properties, namely curvature, morphology, and lateral dynamics, in supported lipid bilayers. Applications of such engineered surfaces in examining the dependence of membrane phase separation and phase transition on bilayer curvature and morphology will be discussed.

8:40am **BI+NC-ThM3 Fluidic and Air-Stable Supported Lipid Bilayer and Cell-Mimicking Microarrays**, ***X.-Y. Zhu***, University of Minnesota

As drug delivery, therapy, and medical imaging are becoming increasingly cell-specific, there is a critical need for high fidelity and high-throughput screening methods for cell surface interactions. Cell membrane-mimicking surfaces, i.e., supported lipid bilayers (SLBs), are currently not sufficiently robust to meet this need. Here we describe a method of forming fluidic and air-stable SLBs through tethered and dispersed cholesterol groups incorporated into the bottom leaflet. Achieving air-stability allows us to easily fabricate SLB microarrays from direct robotic spotting of vesicle solutions. We demonstrate their application as cell membrane-mimicking microarrays by reconstituting peripheral as well as integral membrane components that can be recognized by their respective targets. These demonstrations establish the viability of the fluidic and air-stable SLB platform for generating content microarrays in high throughput studies, e.g., the screening of drugs and nanomedicine targeting cell surface receptors.

9:00am **BI+NC-ThM4 Supported Lipid Membranes as Biomimetic Model Systems**, ***S. Svedhem***, *A. Kunze, H. Ekstrand,* Chalmers University of Technology, Sweden, *P. Sjövall,* SP Technical Research Institute of Sweden, *R. Frost, M. Edvardsson, B. Kasemo*, Chalmers University of Technology, Sweden

Engineering of surface-supported lipid membrane model systems is currently a very active field of research. The present contribution will focus on two recent examples from our group in this area; (i) Lipid exchange between liposomes and supported lipid membranes of opposite charge, and (ii) The action of lipases on supported lipid membrane structures. These examples cover different kinds of supported lipid structures; both (planar) supported lipid bilayers and (intact) supported liposomes, as well as different kinds of biomolecular interactions associated with them. Key experimental techniques used to follow processes at these interfaces are the quartz crystal microbalance with dissipation monitoring (QCM-D), optical reflectometry, surface plasmon resonance (SPR), fluorescence microscopy, atomic force microscopy (AFM) and time-of-flight secondary ion mass spectrometry (TOF-SIMS). Our first examples deals with lipid exchange/transfer between lipid membranes, which is important for many biological functions, but which has also the potential for in situ engineering of supported membranes. To learn more about how the dynamics of such processes can be studied, we have investigated the interaction of positively and negatively charged lipid vesicles with supported lipid bilayers (SLBs) of opposite charge. In particular, it was possible to follow the different steps during such modification processes both by QCM-D and TOF-SIMS, the latter allowing direct estimation of the fraction of different lipids in the membrane. These results have also implications for studies of how nanoparticles interact with membranes. The second example covers how lipases (PLA2 and PLD) act on membranes, and in particular how lag phases for such interactions can be monitored by QCM-D. Depending on the type of lipase under study, either dissolution or membrane morphology changes were observed. In conclusion, the combination of surface-supported lipid membranes and surface-sensitive analytical techniques allows for detailed studies of processes of relevance for biological membranes. In particular, the molecular composition can be controlled, and morphological changes of the membrane structure can be induced and visualized

9:20am **BI+NC-ThM5 Nanopatterning Proteins Over Large Areas for Biological Applications**, *J. Malmström, H. Agheli, P. Kingshott,* ***D. Sutherland***, University of Aarhus, Denmark

The recent decade has seen a rapid expansion in the ability to create and study nanometer scale objects and these new methods are being applied to the study of biological systems. The immobilisation of bioactive molecules has long been a goal in biomaterials and tissue engineering research, for use as stimulatory cues or model systems to study biointeractions. The advent of soft lithographic routes and efficient approaches to minimise non-specific protein interactions for example through immobilised polyethylene oxide coatings has lead to microscale pattens of proteins were routinely demonstrated and applied as model systems to study biological systems. While patterns at the micrometer scale of considerable interest and application, the size of and lengthscale at which proteins and other macromolecules are structured in vivo is in most cases at the nanoscale. Patterning biomolecules at the nanometer scale gives a significant potential for studying how biological systems function at the macromolecular length scale or to mimic the structure of biological interfaces with macromolecular resolution. A key requisite for the study of cellular biosystems is the ability to robustly generate large areas of patterns. In this work colloidal lithographic routes utilising electrostatic self assembly to generate dispersed monolayers of colloidal particles as masks for pattern generation have been used to generate nanostructured interfaces. Substrates with nanopatterned surface chemistry have been used as templates for generation of nanopatterns of proteins. Hydrophobically modified gold nanopatches in a silicon oxide background have been used to open up arrays of 100nm nanometer diameter regions within a protein rejecting background (based on PLL-g-PEG) and used to demonstrate nanopatterning of a number of protein systems (Laminin, Osteopontin and Ferritin). Nanostructured interfaces have also been fabricated on QCM-D sensors and used to study insitu protein and antibody binding at nanoscale patches while AFM microscopy of dried samples was used to quantify protein and antibody binding utilising height histograms. A combination of QCM-D, AFM and SPR derived data was used to establish the thickness and density of the adsorbed laminin layers at both nanoscale patches and homogeneous surfaces.

9:40am **BI+NC-ThM6 Fabrication and Testing of Electrospun Novel Biodegradable Polyurethane Scaffolds**, ***N. Brown***, *C. Zhang, T. Boland*, Clemson University

Synthesis and fabrication of biomaterials that can temporarily mimic the native tissue is a lofty aim in Tissue Engineering. It is also paramount in Regenerative Medicine material research. Such a biomaterial could be formed into scaffolds and be temporary replacements of tissues or for other internal biomaterial corporal needs. Our work here is on the use of a novel biodegradable polyurethane (BPU) that was electrospun and fabricated into tubes. Once fabricated, smooth muscle cells (RASMC) were ink-jet printed onto the same scaffolds and tested for degrees of cell alignment BPU are biopolymers that are designed to mimic the elasticity and memory of native tissue. These biopolymers can be designed to fit the application. This BPU was synthesized from methylene di-p-phenyl-diisocyanate (MDI), polycaprolactone diol (PCL-diol) and N, N-bis (2-hydorxyethyl)-2-aminoethane-sulfonic acid (BES), serving as a hard segment, soft segment and chain extender respectively. The BPU was then electrospun into nanofibers and formed small diameter (4 mm) blood vessels. The blood vessels were electrospun at various extrusion rates to determine optimum pore size and fiber diameters. This was accomplished by SEM imaging. The mechanical testing included tensile and burst pressure testing to determine if the scaffold could withstand extreme physiological conditions. Burst pressure testing results were from 1600-2900 mm Hg. Fiber diameters were in the 700-1000 nm range. Pore sizes were in the 50-90 um range. Mechanical testing results indicated a elongation of 620+/-120% with memory. The mechanical testing indicated that these scaffolds could withstand extreme mechanical physiological conditions well exceeding what they would experience in vivo. The imaging indicated fiber formation that could mimic an extracellular matrix or act as an internal physical barrier. Lastly, ink-jet printing was used as a cell placement method to control the location of cells on material. Cell printing was used to determine if RASMC cell alignment was possible and to what degree patterns could be printed to conjure alignment on the fibrous scaffolds. Histological results of the RASMC patterns on the electrospun scaffolds will be presented.

10:40am **BI+NC-ThM9 Electrically Insulating Pore-Suspending Membranes on Highly Ordered Porous Alumina**, ***C. Steinem***, University of Goettingen, Germany **INVITED**

In the last years, we have developed a membrane system that combines the merits of freestanding and solid supported bilayers. These membranes suspend the pores of a highly ordered porous material such as porous alumina (nano-BLMs) or porous silicon (micro-BLMs). In this talk, I will discuss the electrical properties and stability of these membranes as a function of lipid composition and under flow conditions. We were able to demonstrate that a buffer solution exchange can be readily achieved by placing the membranes in a flow system. The membranes turned out to be stable as evaluated by the changes in membrane resistance obtained from impedance analysis. The membrane resistances are sufficiently high to analyze ion channel activity on the single channel level. In particular, we have demonstrated that connexins can be inserted into nano-BLMs exhibiting full functionality.

11:20am **BI+NC-ThM11 Supported Lipid Bilayers on Nanoporous Substrates for Multi-technique Membrane Sensing**, ***K. Kumar***, *S. Kaufmann, A.M. Tabari, M. Textor, E. Reimhult*, ETH Zürich, Switzerland

Supported lipid bilayers (SLBs) formed from the rupture of liposomes have the advantage over other planar membrane architectures in that they can be formed completely bereft of organic solvents, enabling the further incorporation of sensitive ion channels or membrane proteins.[1] Porous structures allow the use of fusogenic surfaces which enhance the formation of SLBs, while accommodating the incorporation of larger membrane proteins. By integrating these porous structures with suitable gravimetric or optical sensor surfaces that could double up as electrodes, it would be possible to conduct electrochemical measurements in tandem with, e.g., affinity measurements. For optical sensing techniques such as waveguide spectroscopy, if the pores are small enough, it would also be possible to discriminate between processes occurring on the surface and within the pores.[2] We have developed a process where it is possible to etch high aspect ratio pores into silicon nitride and silicon oxide with a tunable diameter between 50 nm and 150 nm using particle lithography for patterning etch masks.[3] Sensor substrates for coupled plasmon waveguide resonance (CPWR) measurements, quartz crystals for quartz crystal microbalance with dissipation (QCM-D) measurements and glass slides for microscopy techniques were fabricated. The influence of nanopore density and size on the kinetics of formation of SLBs by liposome fusion was investigated by QCM-D and the structure of the lipid bilayer in the pore area was investigated by atomic force microscopy, confocal fluorescence microscopy and nanoscopy.[4] QCM-D, microscopy and nanoscopy measurements suggest the formation of fully covering SLBs by liposome fusion on such substrates. Atomic force microscope (AFM) images and force distance measurements on individual SLBs over pores seem to indicate that the formed SLB also spans the nanopores, but are strongly deformed by the tip interaction. These results set the stage for the next phase of experiments, where electrochemical measurements can be made in situ on the waveguide or quartz crystal after the confirmed formation of a pore-spanning SLB.

[1] Reimhult, E. and Kumar, K. TIBTECH, 2008. 26(2): p. 82-89.
[2] Lau, K.H.A., et al. J Phys Chem B, 2004. 108(30): p. 10812-10818.
[3] Reimhult, E., et al. Nanotechnology, 2007. 18(27): p. 7.
[4] Donnert, G., et al. PNAS, 2006. 103(31): p. 11440-11445.

11:40am **BI+NC-ThM12 Patterning of Plasma Polymers for Bioarray**, ***G. Mishra***, *S.L. McArthur*, University of Sheffield, UK

The high-density, multi-anaylate chips required for genomic and proteomic research can be successfully produced using a precise surface patterning methodology that allows controlled positioning of chemically distinct active areas. A major challenge with current bio-sensing devices which requires addressing is the need for surface chemistry that allows immobilised biomolecules of diverse types to retain their biological activity. Plasma polymerisation presents a versatile approach to surface modification of these devices. The range of monomers available for plasma polymerisation makes this manufacturing approach even more suitable for use in systems where multiple coatings with specific properties are required for a single device. The control offered by this surface modification technique and the ability to spatially define reactive regions to reduce non-specific background adsorption is integral to this project. This study highlights the efficacy of photolithographic plasma polymer patterning and provides a rare insight into issues associated with achievable chemical specificity and spatial resolution. A multi-technique investigation (XPS, ToF-SIMS, AFM, fluorescence microscopy) of surface chemistry and its biological response forms the focus of the study. Using ToF-SIMS data and multivariate analysis, we highlight the intricacies of pulsed plasma polymerised surface chemistry and propose a unique approach to optimising these parameters in order to maximise functional group retention. ToF-SIMS data has also been used to provide new insight into the mechanism of pulsed plasma polymerisation.

Biological, Organic, and Soft Materials Focus Topic
Room: 201 - Session BO+EM+BI+NC-ThM

Semiconducting Biointerfaces and Sensors
Moderator: L.J. Brillson, Ohio State University

8:00am **BO+EM+BI+NC-ThM1 AlGaN/GaN HEMT And ZnO Nanorod Based Sensors for Chemical and Bio Applications**, *B.S. Kang, H.T. Wang, K.C. Chen, Y.L. Wang, T. Lele, J. Lin, S.J. Pearton,* ***F. Ren***, University of Florida

AlGaN/GaN high electron mobility transistor based sensors are good candidates for low cost, handheld, and wireless chemical and biomedical sensor due to their excellent thermal as well as chemical stability and sensitivity to the changes of ambient. The electrons in the AlGaN/GaN HEMT two-dimensional electron gas (2DEG) channel are induced by piezoelectric and spontaneous polarization effects and there is no dopant needed. There are positive counter charges at the AlGaN surface layer induced by the 2DEG. Any slight changes in the ambient of the AlGaN/GaN HEMT affect the surface charges of the AlGaN/GaN HEMT. These changes in the surface charge are transduced into a change in the concentration of the 2DEG in the AlGaN/GaN HEMTs. We have demonstrated AlGaN/GaN HEMT based individual sensors for protein, DNA, kidney injury molecules, prostate cancer, pH values of the solutions, pH in the exhaled breath condensate, and mercury ions with specific surface functionalizations. We have also demonstrated ZnO nanorod based sensors to detect UV, hydrogen, carbon monoxide and ammonium. Recently, we integrated ZnO nanorods with AlGaN/GaN HEMT to detect glucose in the breath condensate. This approach makes a possibility of integrating AlGaN/GaN HEMT based sensors with ZnO nanorod sensors on a single "smart sensor chip" with the techniques of selective area functionalization and mircofluidic device approaches. This smart sensor chip can be mounted on a handheld, portable, wireless transmitter circuit board.

8:20am **BO+EM+BI+NC-ThM2 Label-Free Dual Sensing of DNA-Molecules using GaN Nanowires**, ***A. Ganguly***, *C.-P. Chen,* National Taiwan University, *K.H. Chen,* Academia Sinica, Taiwan, *L.C. Chen*, National Taiwan University

GaN, a leading optoelectronic material, is also known to be non-toxic and bio-compatible. Interestingly, this material in the form of nanowires (NWs), with the advantages of large surface-to-volume ratio and direct electrical-path due to surface-induced spatial-separation of charge-carriers, could possess high sensitivity to the local environment, hence to the surface-immobilized biomolecules. Here, we report the GaN NWs possess high bio-binding efficiency and provide a platform for in situ, label-free, and rapid (assay-time within 2 hours) detection of DNA-molecules with dual-sensing capability (electrochemical and optical). Both electrochemical (EC) and optical (photoluminescence, PL) measurements showed clear distinction of pristine GaN NWs with probe-DNA (pLF) immobilization, and after further hybridization, employing a popular target-DNA with anthrax lethal factor sequence (LF). In label-free condition, both EC and PL-based techniques exhibited high sensitivity, without any little effort to optimize the sensing-condition, up to nM and pM of concentrations, respectively for the recognition of LF, with very low assay-time. Furthermore, successful application for detection of "hotspot"-mutations, related to human p53 tumor-suppressor gene, revealed excellent selectivity and specificity towards the fully-complementary targets, down to pM concentration, even in presence of mutations and non-complementary strands, suggesting the potential pragmatic application in complex clinical samples. The simplicity in detection-method, without any requirement of extra step/modification in both probe and target-systems, and simultaneously, the unique label-free dual-detection capability of GaN NWs, with excellent selectivity and sensitivity, can make them a promising choice of transducers, even in clinical application.

8:40am **BO+EM+BI+NC-ThM3 GaN Field Effect Transistors for Biosensor Applications**, ***W. Lu***, The Ohio State University **INVITED**

Biosensors based on electrical field effect transistors (FETs) are of great research interests due to their properties of label free, low cost, small size, and easy integration to external circuitry electronics. Such biosensors have been fabricated on many semiconductor materials including Si, silicon on oxide, carbon nanotube, ZnO, etc. Si-based such biologically FETs (bioFETs) suffer from various difficulties such as limited sensitivity and current drift caused by degradation of gate dielectrics and chemical instability. Due to the chemical inertness and the high concentration of two dimensional electron gas (2DEG) at the AlGaN/GaN interface, AlGaN/GaN heterojunction FETs have great potentials for detection of bioagents in biological buffers with high ionic strengths. In this paper, we will give an overview of recent research progress on GaN FET biosensors. The process

and characterization of functionalization of AlGaN surface for biosensing applications will be discussed. The detection of streptavidin (STA) and monokine induced by interferon γ (MIG) proteins and hybridization process of single strand DNAs by AlGaN/GaN HFETs will be presented. Specifically, for STA detection, at different ionic strengths, the effect of Debye length on detection sensitivity has been demonstrated. No current change is observed for fully biotinylated STA, indicating that there is no non-specific binding. Furthermore, we have used open binding pockets of specifically-bound STA on the biotinylated surface as receptors for detection of biotinylated MIG proteins. The results show that the devices are capable of detecting of biotinylated MIGs at pathological concentrations even at physiological ion strengths. In part, this is due to the superior stability of the AlGaN/GaN HFET platform in buffer, which results in sensor noise being sufficiently low to allow reproducible detection of protein analyte binding. For comparison, regular unbiotinylated MIG proteins gave no current change, indicating that there is no non-specific binding and the change of current is due to the charges transferred from charged analytes.

9:20am **BO+EM+BI+NC-ThM5 Surface Functionalization of ZnO Nanoparticles and Thin Films for Sensor Applications**, ***L. Selegard***, *C. Vahlberg, F. Söderlind, V. Khranovskii, A. Lloyd Spetz, R. Yakimova, P.-O. Käll, K. Uvdal*, Linköping University, Sweden

A new procedure has been developed for functionalization of electrochemically produced ZnO nanoparticles. The core of the particles was characterized using TEM, PEEM and LEEM. Single crystal nanoparticles, with uniform spherical morphology with a size of approximately 50Å were obtained. The first aims of ZnO nanoparticle functionalization were to make a stabilizing molecular layer at the surface and to prepare for further linking possibilities, for use in different types of sensing applications. The functionalization process was investigated and the molecular layer was verifyed by XPS and FT-IR. Parallel studies on biofunctionalization of plane ZnO thin films were performed to obtain a suitable reference system. One of the molecules used for functionalization of the nanoparticle surface was (3-Mercaptopropyl)triethoxysilane (MPTS) as it enables further functionalization on the thiol part and as it has the possibility to form a stable network around the particles. The MPTS linking was investigated by XPS and NEXAFS. The XPS spectra of the functionalized particles showed significant signal from both Si and S verifying the presence of MPTS. XPS core level S2p spectrum further showed presence of SH groups, indicating that thiols was available for further linking processes. Another molecule of interest for nanoparticle functionalization is oleic acid. The strategy is then to coordinate the carboxyl groups to the surface and further linking will be based on hydrofobic interactions. The TEM and PEEM results, so far, indicated that the particles were not fully dispersed but the use of oleic acid showed a much smaller extent of agglomerated particles than for example MPTS. PEEM also showed that the oleic acid capped particles was much more heat stable then MPTS capped once. ZnO nanoparticles show two emission peaks, one band gap related UV-emission and one visible emission arising from oxygen vacancies. In this work fluorescence spectroscopy was used to study the emitted, visible light of the particles as a function of different surface modifications.

9:40am **BO+EM+BI+NC-ThM6 Surface Functionalization and Micropatterning of Ta_2O_5 Films Using Organo-silane and Atom Transfer Radical Polymerization(ATRP) Methods**, ***W. Kulisch***, *D. Gilliland, G. Ceccone, L. Sirghi, F. Rossi, H. Rauscher*, European Commission Joint Research Center, Italy

Optical waveguide biosensors frequently require the use high refractive index thin films such as tantalum pentoxide to act as both as a light guide and as a surface on which active biomolecules can be covalently immobilized. In this work, a process for the room temperature deposition of Ta_2O_5 films onto silicon/silica and thermoplast substrates by reactive ion beam sputtering from a tantalum target has been developed. The resultant high refractive index films have been chemically and optically characterized and methods examined for the wet chemical modification of the oxide film to produce either reactive amino groups or low protein fouling polyethylene glycol (PEG) layers. In both cases the first step toward the functionalization of the as-grown films used either an oxygen plasma or to an UV/O_3 treatment to produce a clean and fully oxidized surface. Using these clean, active surfaces-NH_2 terminated SAMs could be routinely produced using a conventional silanization process using 3-aminopropyl trimethoxysilane (APTMS). To produce high density, low protein binding (anti-fouling) layers a more sophisticated procedure based on surface initiated atom transfer radical polymerization (SI-ATRP) of poly(ethylenglycol)methacrylate (PEGMA) has been used. In this method the oxide surface is firstly modified using a combination of poly(glycidylmethacrylate) (PGMA) and α-bromoisobutyryl bromide (BIB) to produce a surface rich in tethered α-bromoisobutyryl groups. These BIB groups can then used as initiation sites for the growth of dense PEG films using the copper complex catalysed ATRP of PEGMA macromonomers. To characterize the final surfaces and to assist in the optimization of process, time-of-flight secondary ion mass spectrometry (TOF-SIMS), X-ray photoelectron spectroscopy (XPS), contact angle measurements and atomic force microscopy (AFM) have been applied to samples after each step of the different processes. The effectiveness of the two processes will be demonstrated and examples given of methods by which the two methods have been combined for the production of fouling/non-fouling micropatterned surfaces.

10:40am **BO+EM+BI+NC-ThM9 Designing the Interfaces between Biomolecules and Semiconductor Substrates: From the C60 Buckyball Model System to Shape-Restricted DNA Molecules**, ***X. Zhang***, *A.V. Teplyakov*, University of Delaware

A well-characterized interface based upon covalent binding between biomolecules and semiconductor surface was designed using the functionalized self-assembled monolayers (SAM) on Si(111) surface and specific shaped-restricted DNA molecules. This type of interface can serve as a prototype for the future devices in biosensing and single molecule spectroscopy. The spectroscopic and microscopic benchmarks were initially tested using fullerene C60 as a model to understand the attachment chemistry of large molecules with amine-terminated SAM on Si(111) surface. X-ray photoelectron spectroscopy (XPS) and Infrared spectroscopic (IR) studies, supported by computational investigation, verified the covalent attachment of C60 to the amine-terminated SAM on Si(111) surface. The atomic force microscopy (AFM) revealed the topography of the C60-modied surface with molecular resolution. The biomolecule/semiconductor interface was tailored with the same amine-terminated SAM on Si(111) surface and thiol-DNA molecules, which is achieved via a sulfo-succinimidyl 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (SSMCC) crosslinker molecule. The shape-restricted thiol-DNA is anchored to the surface through the formation of covalent bonds as confirmed by XPS and time-of-flight secondary ion mass spectroscopy (ToF-SIMS). The AFM is used to visualize the well-defined and selective covalent binding of thiol-DNA molecules on SAM-covered Si(111). In addition, AFM and contact angle measurement are employed to study the change of the surface topography and the change of the surface hydrophilicity following each step of the DNA attachment chemistry on silicon.

11:00am **BO+EM+BI+NC-ThM10 The Effect of a Spacer Thiol on the Orientation and Hybridization Properties of 40mer DNA SAMs on Gold**, ***L.J. Gamble***, *P.-C. Nguyen, T. Weidner*, University of Washington, *D.W. Grainger*, University of Utah, *D.G. Castner*, University of Washington

Methods for surface-immobilizing single-strand nucleic acids while preserving their original hybridization specificity with minimal non-specific interactions remain an important goal for improving the performance of surface bound DNA microarray and biosensor applications. Before the full potential of DNA microarrays can be realized, fundamental issues must be better understood, including how the crowding, conformation and orientation of immobilized DNA impacts DNA target hybridization efficiency. For detection of small amounts of DNA in a target solution, the coverage and orientation of DNA probes should be optimized for the capture of low concentrations of DNA via hybridization. In this study the effect of backfill of mercaptohexanol (MCH) on 40mer thiolated single stranded DNA (SH-ssDNA) sequence orientation and hybridization efficiency is studied with X-ray photoelectron spectroscopy (XPS), near-edge X-ray absorption fine structure (NEXAFS) spectroscopy, sum frequency generation (SFG) vibrational spectroscopy, and surface plasmon resonance (SPR). While XPS and NEXAFS are both ultra-high vacuum techniques, SPR is a real-time solution based technique and SFG spectroscopy can be acquired both at the solid-air interface as well as the solid-liquid interface. While the 40mer DNA is longer and expected to be more disordered, the NEXAFS N k-edge spectra showed increased the polarization dependence of the peaks at 401eV and 399eV for the 40mer sequence increased with an MCH backfill time of 1 hour. SFG spectroscopy results showed that incorporation of the MCH into the DNA monolayer resulted in an increase of the nucleotide related peaks at 2961 cm^{-1} and 3045 cm^{-1} which maximized at 1 hour MCH backfill. This evidence of orientation changes in the surface bound 40mer DNA will be correlated with hybridization data from SPR.

11:20am **BO+EM+BI+NC-ThM11 Anchoring and LT-STM/STS Characterization of Single Organic Molecules at Semiconducting and Insulating Surfaces**, ***M. Szymonski***, *A. Tekiel, S. Godlewski, G. Goryl, J. Prauzner-Bechcicki, J. Budzioch*, Jagiellonian University, Poland

In recent years self-assembling of organic molecules deposited onto different surfaces have attracted considerable attention because of important applications in organic electronic technologies and prospects for

development of single molecule computing devices. In this presentation we will report on our recent studies of initial stages of growth and organization of several organic molecules on reconstructed (001) surface of InSb, (011) and (110) surfaces of TiO2, and on ultrathin KBr layers grown epitaxially on InSb(001). Among different organic molecules the perylene derivative - 3,4,9,10-perylene-tetracarboxylic-dianhydride (PTCDA), often regarded as a model system for planar-stacking molecules, copper phthalocyanine (CuPc), violet landers (VL), and chiral helicene[11] molecules were studied by means of scanning tunneling microscopy (STM) at room and liquid nitrogen temperatures, nc-AFM at room temperature and low energy electron diffraction (LEED). It is shown that during initial stages of growth on InSb molecules often form chains parallel to [110] crystallographic direction of the c(8x2) reconstructed substrate. They are frequently attached to the lower terrace step edge, or some defects on the surface, indicating surprisingly weak interaction between the molecules and the substrate and their high mobility along [110] diffusion channel. Geometrical orientation of the molecules with respect to the reconstruction rows of the substrate will be discussed. We will present the images acquired with submolecular resolution, as well as images demonstrating the pinning effect of the molecular chains by surface charge density waves, and incorporation of the molecules into overall electronic structure of the system.

11:40am **BO+EM+BI+NC-ThM12 Molecular Self-Assembly of Funtionalized Fullerenes on a Closed Packed Metal Surface**, ***B. Diaconescu***, University of New Hampshire, *T. Yang*, *S. Berber*, Michigan State University, *M. Jazdzyk*, *G. Miller*, University of New Hampshire, *D. Tomanek*, Michigan State University, *K. Pohl*, University of New Hampshire

Self-assembled organic thin films have a great number of practical applications, ranging from sensors and biological interfaces in medical implants to organic electronics and photovoltaics. Generally speaking, self-assembled monolayers (SAMs) form as a result of a delicate balance between competing molecule–substrate and intermolecular interactions. Therefore, to control such self-assembly processes in a useful way, it is mandatory to understand how this balance reflects onto the SAM's final structure. Here, we present a combined STM and DFT study of the self-assembly of C60 molecules functionalized with long alkane chains, F-C60, on the (111) surface of silver. We find that F-C60 molecules lay down on the Ag surface and form a complex zigzag like pattern with an oblique unit cell of 4 nm by 2.5 nm and two molecules per basis. The C60s are placed at a larger than van der Waals distance. The symmetry of the functionalized C60 self-assembled monolayer is dictated by the alkane–surface interaction while the size of the unit cell is a consequence of the in-plane intermolecular interactions. These results show that C60s can be assembled in a two-dimensional and non-compact molecular array and suggest a way to control their surface density via appropriate chemical functionalization.

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Biomaterial Interfaces
Room: 202 - Session BI+TF+MI+NS+NC-ThA

Plasmonics and Magneto/Plasmonics Aimed at Biosensing
Moderator: F. Höök, Chalmers University of Technology, Sweden

2:00pm **BI+TF+MI+NS+NC-ThA1 Optical Meta Materials and Nano Plasmonics**, ***X. Zhang***, University of California, Berkeley **INVITED**
Recent theory predicted a new class of meta structures made of engineered sub wavelength entities - meta "atoms" and "molecules" which enable the unprecedented electromagnetic properties that do not exist in the nature. For example, artificial plasma and artificial magnetism, and super lens that focuses far below the diffraction limit. The metamaterials may have profound impact in wide range of applications such as nano-scale imaging, nanolithography, and integrated nano photonics. I'll discuss a few experiments that demonstrated these intriguing phenomena. We showed, for the first time, the high frequency magnetic activity at THz generated by artificially structured "meta molecule resonance", as well as the artificial plasma. Our experiment also confirmed the key proposition of super lens theory by using surface plasmon. We indeed observed optical superlensing which breaks down so called diffraction limit. I'll also discuss nano plasmonics for imaging and bio-sensing. The surface plasmon indeed promises an exciting engineering paradigm of "x-ray wavelength at optical frequency".

2:40pm **BI+TF+MI+NS+NC-ThA3 Gold and Silver Nanocrescents as Tunable Substrates for Surface Enhanced Infrared Absorption Spectroscopy**, *R. Bukasov*, ***J.S. Shumaker-Parry***, University of Utah
Controlling the size, shape, and orientation of metal nanoparticles in order to tune and optimize the particles' optical properties for specific applications remains a challenge in the field of plasmonics. Tuning the localized surface plasmon resonance (LSPR) wavelength as well as the localized field enhancements is especially important for spectroscopy applications such as surface enhanced Raman spectroscopy (SERS) and surface enhanced infrared absorption spectroscopy (SEIRA). Although SERS has receive a lot of attention with the engineering of nanoparticle-based substrates, the activity in SEIRA development has been less, most likely due to the lack of tunable substrates for the IR spectral region. We describe the development of gold and silver nanocrescents as tunable substrates for SEIRA studies. We use nanosphere template lithography to fabricate gold and silver crescent-shaped structures which exhibit multiple, polarization-sensitive plasmon resonances that are tunable from the visible through the infrared. Large electromagnetic field enhancements are expected due to the sharpness of the crescent's tips and the ability to bring these sharp tips into close proximity to each other. Using the crescent-shaped structures as substrates, we demonstrate the importance of spectral tunability for maximizing signal enhancements in SEIRA. The nanocrescent area normalized SEIRA signal enhancement increases from 7,700 to 46,000 with an increase in the extent of overlap of the nanocrescents' LSPR frequency with the frequency of the probed molecular vibration. The broad tunability of the nanocrescents' LSPR properties makes the structures excellent candidates for a range of spectroscopic and sensing applications including SEIRA.

3:00pm **BI+TF+MI+NS+NC-ThA4 Use of Angle-Resolved SPRi for the Characterization of Protein Binding and Agglomeration Dynamics**, ***M.S. Golden***, *J.A. Ruemmele, A. Whitty, R.M. Georgiadis*, Boston University
Transient protein-protein interactions are essential on almost every level of cellular function. In addition, protein aggregates play various roles in cell signaling pathways and have been implicated in the onset of many neurodegenerative conditions such as Alzheimer's disease. Although various structures of protein complexes have been widely studied, the mechanisms involved in protein binding events are not clearly understood, and the transient dynamics of this formation have proven difficult to study. Investigation of the mechanisms of protein agglomeration and binding, however, is essential to elucidating the role of these structures in diseases. Here we exploit the multi-array quantitative capabilities of angle-resolved surface plasmon resonance imaging (SPRi) to perform kinetic and thermodynamic measurements of protein-protein interactions. Specifically, the agglomeration and small molecule inhibition of Tumor Necrosis Factor (TNF) family members whose primary role is the regulation of immune cells is under investigation. Surface fabrication techniques coupled with multi-channel microfluidic delivery will be employed in order to introduce protein binding partners and small molecules to the surface. Effects of density, orientation, and heterogeneity of surface immobilized protein molecules on protein binding efficiency and kinetics will be investigated and optimum surface fabrication conditions will be identified. In addition, a unique multi-wavelength SPRi approach will be implemented to simultaneously determine dielectric constants and thicknesses of protein layers on a surface. These studies will therefore allow aggregate and nonaggregate structures at the surface to be clearly differentiated. The results of these fundamental studies will allow a broader understanding of how proteins act cooperatively.

3:20pm **BI+TF+MI+NS+NC-ThA5 Sensitivity Enhancement of Surface Plasmon Resonance Imaging by Nanoarrayed Organothiols**, ***P. Lisboa***, *A. Valsesia, I. Mannelli, P. Colpo, F. Rossi*, JRC-European Commission, IHCP, Italy
The implementation of sensor platforms providing high sensitivity of detection is a crucial step for the design of the new analytical device generation for biosensor developments. Designing platform with active/non-actives region at nanoscale has shown already a drastic increase of detection sensitivity.[1,2] The use of organothiols to create nanopatterns has been already studied showing that this type of chemistry is indicated to produce chemical contrasts for bio-applications.[3] In this work, the effect of organothiols-nanopattern in Surface Plasmon Resonance imaging (SPRi) detection was studied. The gold surface of the SPRi chip was divided in two areas, one modified with a chemical nano-contrast based in two different organothiols (thiolated polyethylene oxide (PEO) and Mercaptohexadecanoic acid (MHD) and the other modified uniformly with MHD. The SPRi study was based on the detection of the immunoreaction between Human IgG and anti-Human IgG (Ab specific) by comparing the results obtained with nanostructured and uniform carboxylic surface. First Human IgG was immobilised on the chip and after the recognition of different concentrations of anti-Human IgG was realised. The achieved SPRi signal was higher in the case of the nanostructured areas for all the tested concentrations. Since the active surface with carboxylic functionalities presented only 3% of the total area, one would expect the detection signal to be 3% of the signal obtained for the uniform MHD surface. The fact that the signal from the nanostructures is higher than in the MHD surfaces in SPRi detection is related to an amplification of signal due to the 2D crystalline character of the structures. This type of arrangement presents the geometry of a photonic crystal leading to the interaction between the Surface plasmon polaritron modes and the regular modulation of the dielectric constant of the surface above the gold film modifying the plasmon effect and consequently increasing the measured reflectivity. These results indicate that SPRi detection performance can be improved by the rational functionalisation of the prism surface with 2D crystalline nanopatterns. Moreover adhesive – nonadhesive nanopatterns are recognized to be good platforms for the correct immobilization of the biomolecules on biosensing surfaces.

References
[1]K. Lee, et al., Nano-Letters, 2004 4, 1869.
[2]A. Valsesia, et al., Langmuir 2006, 22, 1763.
[3]P. Lisboa, et al., Applied Surface Science, 2006, 253, 4796.

4:00pm **BI+TF+MI+NS+NC-ThA7 New Developments in Magneto-Plasmonic Devices**, ***C. Clavero***, *J.R. Skuza, K. Yang, R.A. Lukaszew*, College of William and Mary **INVITED**
In recent years Surface Plasmon Resonance (SPR) sensors have been extensively used in bio-sensing applications. SPR is a charge density oscillation at the boundary between a metal and a dielectric material that gives rise to highly confined fields at the interface. As a consequence the SPR excitation condition is very sensitive to changes in refractive index in the dielectric medium and hence to bio-molecules adsorbed at the metal surface. Nevertheless, the required detection sensitivity for low concentrations of small molecules exceeds current SPR sensors. A new kind of sensors combining plasmonic and magneto-optical (MO) properties, i.e. magneto-plasmonic devices, is being actively investigated. In particular, Au-Co-Au trilayers have been found to increase sensitivity in this type of biosensors.[1] This is due to MO activity enhancement in the Co film caused by the high electro-magnetic field created by SPR.[2] We will report on Au-Co-Au thin film tri-layers grown on glass using UHV magnetron sputtering. The optimization of growth conditions and accurate control of films thickness is critical to achieve a remarkable increase in MO activity and hence in overall sensitivity. A practical issue in these multilayer systems is the bad adhesion of Au to glass causing degradation of the sensor when exposed to a water flux. We will show how to circumvent this problem by inserting Cr or Ti thin buffer layers. A different approach to magneto-plasmonic materials, namely fabrication of Au-Co nano-composite

materials in thin film form, will also be presented. These materials are expected to exhibit enhanced MO response due to localized surface plasmon resonances (LSP) within the Co nano-particles and also considerable reduction of light absorption associated with ferromagnetic materials thus increasing the overall sensitivity of the bio-sensor. Parameters such as shape, size and inter-particle distance can be tuned to control the optical and magnetic properties of the material. Au-Co nano-composite materials in thin film form were obtained by magnetron sputtering co-deposition of Au and Co where parameters such as Au and Co concentration, deposition temperature and film thickness were accurately controlled. Finally, different optical configurations for the excitation of the surface plasmon resonance will be discussed.

[1] B. Sepulveda et al. Opt. Lett. 31, 1085 (2006).
[2] V. I. Safarov et al. PRL 73, 3584 (1994).

4:40pm **BI+TF+MI+NS+NC-ThA9 Grafting Thermoresponsive Polymers on Gold Nanoparticles with Atom Transfer Free Radical Polymerization**, ***S. Chakraborty***, *V.H. Perez-Luna*, Illinois Institute of Technology

Thermoresponsive polymer brushes on colloidal gold were formed through Atom Transfer Free Radical Polymerization (ATRP) of N-isopropylacrylamide (NIPAAM) in aqueous media. In this approach, the "grafting from" technique was used with Atom Transfer Radical Polymerization (ATRP) to grow polymer chains from the surface of gold nanoparticles (~20nm). 'Grafting from' using the ATRP technique enables more uniform/homogenous coverage of polymer chains on the surface of gold nanoparticles. Other advantages of ATRP are the growth of polymer chains without chain termination or chain transfer and that the presence of an active initiator site at the end of the growing polymer chain facilitates synthesis of block copolymers. In the present work, PNIPAAM was grown from the surface of nanoparticles with the help of 2-bromopropionyl bromide as the initiator. The reaction was carried out at room temperature under inert atmosphere and aqueous conditions. The system was found to exhibit thermoresponsive behavior with increase in temperature above 32oC. This behavior could be exploited to develop aggregation based assays. The hybrid polymer-gold nanoparticle system was characterized using Optical Absorption Spectroscopy, Fourier Transform Infra-Red Spectroscopy (FTIR) and Dynamic Light Scattering (DLS). These analytical techniques confirmed the growth of polymer chains in the reaction scheme yielding the final product. The ability to make block copolymers with this metal-polymer hybrid system opens up a wide range of applications such as drug delivery systems, detection assays and bioseparations.

5:00pm **BI+TF+MI+NS+NC-ThA10 Reconfigurable Core-satellite Nanoassemblies as Molecularly-Driven Plasmonic Switches**, *D.S. Sebba, J.J. Mock, D.R. Smith, T.H. LaBean,* ***A.A. Lazarides***, Duke University **INVITED**

Colloidal metal nanoparticles support localized surface plasmon resonances that are sensitive to the presence of molecules, materials, and other polarizable particles that assemble in their near fields. Biomolecule nanoparticle conjugates have been implemented in various molecular detection applications in formats that allow monitoring of plasmonic response. Each specific format has vulnerabilities as well as advantages. For instance, monolayers of immobilized particles functionalized with receptors respond sensitively to target molecules and can be used to track kinetics, but are equally sensitive to non-specific adsorbates, a disadvantage shared with traditional, thin film surface plasmon resonance (SPR). Other formats, such as target induced particle aggregation offer strong plasmon modulation, but involve a complex bulk phase process that presents a significant barrier to quantitative interpretation of the optical data. Here, we report plasmon modulation in pre-formed, few particle assemblies linked by reconfigurable DNA nanostructures. The investigation is motivated by the potential of reconfigurable few particle assemblies to provide control of plasmon coupling, and ultimately, to yield a signal that is distinguishable from plasmonic variations associated with non-specific interactions. In the coupled system upon which we report, DNA nanostructures tether satellite particles to a core particle of like or unlike composition. The DNA nanostructures use duplex DNA to control interparticle separation and are responsive to target strands that modulate interparticle helix length. The reconfigurable assemblies are characterized structurally using dynamic light scattering and transmission electron microscopy and optically using elastic scattering spectroscopy. We demonstrate that DNA nanostructures provide molecular control of interparticle separation by correlating measured plasmonic signals with simulated signals derived from models based upon measured structural parameters. In order to study the sensitivity of core-satellite spectral response to colloid material properties, single assembly scattering spectroscopy and multi-color CCD image analysis are used to monitor perturbation of the core plasmon resonance induced by assembly of satellites of various composition.

Biological, Organic, and Soft Materials Focus Topic
Room: 201 - Session BO+NS+BI+NC-ThA

Biological and Molecular Applications of Nanostructures
Moderator: P. Kingshott, The University of Aarhus, Denmark

2:00pm **BO+NS+BI+NC-ThA1 Fabrication of Nanoscale Bioarrays for the Study of Cytoskeletal Protein Binding Interactions Using Nano-Imprint Lithography**, ***M. Schvartzman***, *M. Palma, J. Abramson, J. Sable, J. Hone, M.P. Sheetz, S.J. Wind*, Columbia University

Recent advances in solid-state nanofabrication technology now make it possible to fabricate structures in the size regime of biomolecules, i.e., ~ tens of nanometers and below. We are developing a system that mimics biological spatial order by using nanofabricated structures which are organized into hierarchical arrays in which structural parameters, such as spacing and orientation, are systematically varied, and which provide multiple protein binding sites with nanometer-scale separations. The aim of the work is to study the dependence of large cytoskeletal protein binding on the geometrical arrangement of extracellular matrix (ECM) proteins and integrins. Nanoscale patterns are formed in arrays containing metal dots 5 - 10 nm in diameter, which are functionalized with linker molecules that specifically interact with individual protein binding sites. These dots can be arranged individually, in pairs, or in more complex patterns based on the structure of the molecules under investigation. In particular, we are interested in understanding of the importance of the spacing between integrin cytoplasmic tails on the binding of other proteins, such as talin, that are involved in the building of focal adhesion (FA) complexes by which the actin cytoskeleton attaches to the ECM. The nano-arrays fabrication process uses thermal nanoimprint lithography and pattern transfer by Au/Pd deposition and lift-off. For the lift-off process for such small features and relatively thin resist layer, an angle evaporated metal hard mask is deposited after the NIL step, followed by resist descum. A post-lift-off annealing step at 400 – 500 oC results in further reduction of feature size and a high degree of uniformity. Spheroidal dots are formed with diameters ~5 - 10 nm. The pattern is functionalized with fibronectin RGD motif through a biotin-avidin-biotin linkage. Total-Internal-Reflectance Fluorescence (TIRF) is used for the monitoring of the bio-functionalization with fluorescene labeled molecules. In-vitro study of cells spreading on the patterned and bio-functionalized surfaces is performed on the patterns with different geometries. This presentation will describe the fabrication arrays of ultra-small metal features using NIL technology, functionalization and implementation of these arrays in the study of the fundamentals of cell behavior, representing a new example of the enormous impact of nanofabrication on the life sciences.

2:20pm **BO+NS+BI+NC-ThA2 Universal Method for Forming Various Metal Particles as Multiplexed Labels for Electron Microscopy in the Backscattering Mode**, ***H. Takei***, *H. Kim, K. Yasuda*, Kanagawa Academy of Science and Technology, Japan

Scanning electron microscopy is a powerful technique in terms of resolution, three dimensional rendition of the object and use of ease. Life science is one among many fields for which SEM is an indispensable tool, and it continues to find new uses. One way to enhance its utility in the field of life science is to multiplex labeling as carried out routinely with fluorescence microscopy; labels with different emission spectra. For this purpose, one approach would be to use particles made from different metals because different metals backscatter electrons differently in accordance to the atomic weight. In the backscattering mode, particles made from different metals can be readily distinguished by the brightness of the image so that labeling each type of the particle with distinct biomolecules such as an antibody or DNA would allow one to observe distributions of distinct molecular species simultaneously, as with multiplexed fluorescence labeling, albeit at much higher resolutions. What is needed is thus a method to produce various metal particles at will. For this purpose, we will demonstrate a universal method consisting of formion of a dense monolayer of monodisperse Latex spheres with a self-assembling technique and then evaporating a metal that can be readily evaporated or sputtered. With the above method, the sphere adsorption is accomplished through mild physisorption so that application of mild sonication to surface bound metal particles in the presence of a liquid such as water leads readily to re-dispersal of Latex sphere coated with the metal. We show that resulting particles made from different metals such as gold, silver, nickel or aluminum can be readily distinguished. The sphere size used ranges typically from 50 to 100 nm with the deposition thickness anywhere between 5 to 20 nm. Beside its flexibility with respect to the type of metal that can be used, another advantage of this method lies with a fact that particles can be surface-modified while still adsorbed on a surface. This is

particularly advantageous from the perspective of rinsing after each surface treatment protocol. It should be mentioned that because particles are covered only on one side, two types of surfaces, metal and exposed polystyrene, can be selectively used for surface modifications; if necessary two sides can be modified with two distinct species at the same time to give higher functionalities.

2:40pm **BO+NS+BI+NC-ThA3 Nanoscopic Presentation of Peptides at Cell Interfaces**, *J.P. Spatz,* ***V. Hirschfeld-Warneken***, MPI for Metals Research and U. of Heidelberg, Germany **INVITED**

Engineering of cellular environments has become a valuable tool for guiding cellular activity such as differentiation, spreading, motility, proliferation or apoptosis which altogether regulates tissue development in a complex manner. The adhesion of cells to its environment is involved in nearly every cellular decision in vivo and in vitro. Its detailed understanding and defined control also opens new strategies for medical technologies with respect to, e.g., stem cell regulation, tissue scaffolds, cell selection due to their disease state, artificial blood vessels, or immunology. Our approach to engineer cellular environments is based on self-organizing spatial positioning of single signaling molecules attached to inorganic or polymeric supports, which offers the highest spatial resolution with respect to the position of single signaling molecules. This approach allows tuning cellular material with respect to its most relevant properties, i.e., viscoelasticity, peptide composition, nanotopography and spatial nanopatterning of signaling molecule. Such materials are defined as "nano-digital materials" since they enable the counting of individual signaling molecules, separated by a biologically inert background. Within these materials, the regulation of cellular responses is based on a biologically inert background which does not trigger any cell activation, which is then patterned with specific signaling molecules such as peptide ligands in well defined nanoscopic geometries. This approach is very powerful, since it enables the testing of cellular responses to individual, specific signaling molecules and their spatial ordering. Detailed consideration is also given to the fact that protein clusters such as those found at focal adhesion sites represent, to a large extent, hierarchically-organized cooperativity among various proteins. Moreover, "nano-digital supports" such as those described herein are clearly capable of involvement in such dynamic cellular processes as protein ordering at the cell's periphery which in turn leads to programming cell responses.

3:20pm **BO+NS+BI+NC-ThA5 Bioadhesive/Bioresistant Nanopatterns: Fabrication, Characterization and Investigation of Their Effect on Enhancing Biomolecule Affinity Reactions**, ***I. Mannelli,*** *A. Valsesia, P. Lisboa, P. Colpo, F. Rossi*, European Commission Joint Research Centre, Italy

Fabrication and characterisation of nanopatterned surfaces are topics of many past and present research studies. Recently many techniques have been investigated for the fabrication of nanopatterned surfaces. Among them colloidal lithography combined with plasma polymer deposition and/or self-assembly has shown to be a flexible technology for producing large area nanostructured surfaces. At the same time investigations have increasingly focused on the behavior of biomolecules (i.e. protein, nucleic acid) when interacting with surfaces and/or particles with nanometre dimensions. Although many studies have been done on the surface/biomolecule interaction mechanisms and the conformation changes that molecules undergo after the interaction with macroscopic surfaces it is much less well understood how the biomolecules interact with surfaces that have been previously functionalised with nanofeatures. In particular little is known about the interactions which occur with features with dimensions comparable with those of the biomolecules themselves and how, after the interaction, the native molecule conformation changes. We have optimized a procedure for fabricating nanopatterned surfaces at the sub500 nm scale in which a hexagonal close packed array of bioadhesive gold nanoareas are embedded in an anti-fouling matrix (PEO-like polymer). The surfaces fabricated in this way were characterized by AFM analysis and their interaction with amino functionalised Au nanoparticles were investigated. The AFM images show the crystalline arrangement of nanopattern array and the localization of the H_2N-Au nanoparticles in the bioadhesive areas. A SPR Imaging system was used to perform kinetics studies on the adsorption and interaction behaviour of biomolecules on these surfaces. At the same time, the detection performance of these surfaces when employed as a transduction platform for studying biomolecule interactions has been investigated. To do this, a recognition biomolecule was immobilized on the surface and the affinity reaction with a specific target molecule was monitored in real time by means of the SPRi system. The investigated surfaces showed an enhancement of the affinity reaction efficiency with respect to the non structured surfaces. The results obtained show that nanostructuring the surfaces makes it possible to improve the binding site accessibility of the immobilized biological probes without significantly modifying the native biomolecule conformation.

4:00pm **BO+NS+BI+NC-ThA7 Control and Separation of Proteins in a Nanofluidic FET Device, using pH Gradient and Valence Charge**, ***Y.-J. Oh,*** University of New Mexico, *D.R. Bottenus, C.F. Ivory,* Washington State University, *S.M. Han*, University of New Mexico

We have fabricated Si multiple internal reflection infrared waveguides embedded with a parallel array of nanofluidic channels (100 nm W ×500 nm D) and studied field-effect-transistor (FET) flow control and separation of proteins, using scanning laser confocal fluorescence microscopy (SL-CFM) and multiple internal reflection Fourier transform infrared spectroscopy (MIR-FTIRS). For fluidic FET, a DC potential is applied to a highly doped gate area in the mid-section of nanochannels, in addition to a longitudinal electric field along the nanochannels. The gate potential controls the surface charge on SiO_2 channel walls and therefore their ζ-potential. Depending on the polarity and magnitude, the gate potential can accelerate, decelerate, or reverse the flow of proteins. In addition, our MIR-FTIR analysis demonstrates that fluorescein dye molecules, used here as a pH indicator, are hydrogenated and dehydrogenated in response to the gate bias and subsequent pH shift. Using fluorescein, we have thus measured a pH shift caused by the surface charge modulation and longitudinal electrical field. We observe that this pH shift is further influenced by water electrolysis occurring at the electrodes that drive the electroosmotic flow as well as at the gate where a leakage current unavoidably flows through a thermal SiO_2 layer. Using this pH manipulation and generating a pH gradient along the nanochannels, we have conducted isoelectric focusing and separation of proteins with different isoelectric points (Ip). In this presentation, we will further discuss protein separations, using transverse electromigration based on their different valence charges in relation to the surface charge on channel walls.

4:20pm **BO+NS+BI+NC-ThA8 High Throughput Device for Surface Modification Studies**, ***S. Saxer,*** *S. Tosatti, S. Zuercher,* ETH Zurich, Switzerland, *K. Gademann,* EPF Lausanne, Switzerland, *M. Textor*, ETH Zurich, Switzerland

Surface coatings are often used to control the degree of the interaction between the material and the surrounding environment. Paints and lacquers are the most common and widespread coatings. Recent developments in the process of micro- and nanostructures lead to the demand of thinner coatings, which maintain the aspect ratio and preserve the fine structures. Such conditions can be fulfilled by the use of self-assembled monolayers.[1] Due to the large spectrum of applications are nowadays high-throughput approaches required to screen through a large number of parameters, ranging from the substrate up to the different assembly conditions (temperature, solvent, pH). We designed a surface modification screening platform (SuMo-device), which allows the parallel execution of different surface–modification experiments while allowing a single measure for the adlayers characterization, thus enhancing the efficiency of the experiment. The seventy wells (working area/well: 7mm^2; Volume/well: 20μmL) provided by the SuMo-device, were verified with solutions having different concentrations of the grafted copolymer; poly (L-lysine)-grafted-poly ethylene glycol, which is known to adsorb electrostatic to different metal oxide substrates and to render the surface resistant against non-specific protein adsorption (non-fouling), and thus resulting in adlayers with different coverage and thickness.[2] A secondary adsorption of fluorescein isothiocyanate labeled fibrinogen (FITC-fbg) enables to test the non-fouling behavior and therefore the quality of the polymer layer, by the measurement of the fluorescence with a microarray scanner.[3] A standard evaluation procedure was introduced to enable a quantification of the fluorescent response. The Limit of Detection (LOD) was calculated from the standard curve and requires a minimum FITC-fbg concentration of 0.002mg/mL (incubation: 1 h at 25°C). The fluorescence data were compared and found to correlate with layer thickness (ellipsometry) and with the in situ mass adsorption curve obtained by optical biosensor devices. Thus, we conclude that our approach offers a faster and more efficient way to screen between different possible coatings strategies, similarly to drug discovery processes.

[1] F. Schreiber, Prog. Surf. Sci. 65, 151 (2000)
[2] G.L. Kenausis et al., J. Phys. Chem. B 104, 3298 (2000)
[3] S. Pasche et al., Langmuir 19, 9216 (2003).

4:40pm **BO+NS+BI+NC-ThA9 Nanopatterned Functional Surfaces by Electron Beam Lithography of Plasma Assisted Chemical Vapour Deposited Polymers**, *F. Bretagnol, A. Valsesia,* ***G. Ceccone,*** *D. Gilliland, P. Colpo, F. Rossi*, European Commission, Joint Research Centre Italy

We presents a method for the fabrication of well defined chemically active nano-patterned surfaces. Electron-beam lithography is applied on plasma-deposited polymers in order to create sub-micron carboxylic functional areas over a non-bio-adhesive matrix. Characterization of the patterned surface demonstrates that this technique can be used to fabricate chemically active features with lateral size down to 200 nm. Moreover, experiments with a model protein (bovine serum albumin) on the patterned surfaces

show preferential adhesion on the active region indicating the ability of this method for the design of biosensing platforms.

5:00pm **BO+NS+BI+NC-ThA10 Application of Plasma Polymerised Microchannel Surfaces for IEF**, ***M. Salim***, *B. O'Sullivan, S.Y. Ow, P.C. Wright, S.L. McArthur*, The University of Sheffield, UK

The emerging proteomics field has triggered the development of many bioanalytical tools and technologies. Since biological samples are usually present in small quantities and volume, there is a high priority for the analytical tools to be able to process these minute samples. The use of microfluidics for this purpose has thus emerged. Plasma polymerisation has been shown to be an attractive method to coat microchannel surfaces. Here, we apply the plasma surface modified microchannels for on-chip IEF protein separation (separation based on the isoelectric points of the proteins). A common problem associated with the use of non-coated glass or fused silica substrates in IEF is the high electroosmotic flow (EOF) and high extent of protein adsorption. Therefore, coated microchannel surfaces are usually introduced to reduce the EOF and reduce protein adsorption, and improve the resolution of the separation. Lower EOF can also permit IEF separation in short channel lengths. In this study, we investigate the applicability of plasma polymerised tetraglyme surface for IEF-facilitated protein separation at a 2.2 cm channel length, since it exhibits slower EOF compared to non-coated microchannel surfaces, and is low-fouling. Comparisons were also made to non-coated microchannels and other types of plasma polymers, i.e. plasma polymerised acrylic acid and plasma polymerised allylamine coated surfaces. IEF performed on the low-fouling plasma polymerised tetraglyme microchannel surface was shown to exhibit better separation compared to non-coated microchannel, as observed from the clearer formation of focused protein bands. Also, due to the presence of protein and carrier ampholytes adsorption in both plasma polymerised acrylic acid and allylamine coated microchannels, these surfaces may not be suitable for IEF protein separation over a wide range of pH, with the separation resolutions decreasing after repeating experimental runs.

5:20pm **BO+NS+BI+NC-ThA11 Plasma Deposited Polyethylene Oxide as a Platform for Proteins and Stem Cells Microarrays**, *L. Ceriotti,* JRC, Ispra, Italy, *L. Buzanska,* JRC, Ispra, Italy and Polish Academy of Science, *H. Rauscher, I. Mannelli,* ***L. Sirghi****, D. Gilliland, M. Hasiwa, F. Bretagnol, A. Ruiz, S. Bremer, S. Coecke,* JRC, Ispra, Italy, *P. Colpo,* Joint Research Center, IHCP, Italy, *F. Rossi*, JRC, Ispra, Italy

In this work we fabricated and characterized microarrays of proteins of the extra cellular matrix (ECM) for stem cells adhesion studies. Plasma deposited poly(ethylene) oxide (PEO-like) film coated glass slides has been chosen for its dual properties, being protein and cell repellent in wet conditions and protein adhesive in dried conditions. The microarrays were created by direct microspotting of the proteins on the PEO films with optimized printing buffer by using a non-contact printing technology. The stability and the quality of the spots of fibronectin used as model protein were assessed by Time of Flight- Secondary Ion Mass Spectrometry (ToF-SIMS) and ellipsometry was used to determine the amount of protein immobilized on each spot after rinsing of the substrate with water. It was found that when fibronectin is spotted at a concentration higher than 84 μmg/ml, the protein forms a monolayer with a density of 112 ± 4 ng/cm^2 with a low surface coverage but quite regular spatial distribution as confirmed by Atomic Force Microscopy (AFM) measurements. The active conformation of the spotted fibronectin as a function of the spotted concentration was verified by performing an immunoassay with antibodies specific for the fibronectin RGD sequence by Surface Plasmon Resonance (SPR) imaging. Human Umbilical Cord Blood Neural Stem Cells (HUCB-NSCs) were cultured on different ECM protein arrays (fibronectin, laminin, collagen I, collagen III and collagen V) showing a protein type and concentration dependent adhesion and growth on the micro-spots. No cells were found in-between the spots thanks to the anti adhesive properties of the PEO-like film. The cell nuclei were stained for cell counting and preliminary specific cell staining was performed to evaluate the differentiation stage of HUCB-NSCs on fibronectin spots. The array platform developed in this study provides a promising approach to investigate in a high throughput manner how insoluble factors patterned on the surface influence stem cell adhesion and development.

Biomaterial Interfaces
Room: Hall D - Session BI-ThP

Biomaterial Interfaces Poster Session with Focus on Engineered Bio-Interfaces and Sensors

BI-ThP2 High-Sensitivity Surface Enhanced Raman Scattering of Sub-Picomole Level of Adenine and Thymine Species at Au/Ag Nanoparticle Modified Silicon Nanotip Arrays, ***H.C. Lo***, National Chiao-Tung University, Taiwan, *H.I. Hsiung*, National Taiwan University, *Sv Chattopadhyay*, National Yang-Ming University, Taiwan, *C.F. Chen*, Ming-Dao University, Taiwan, *J. Leu*, National Chiao-Tung University, Taiwan, *K.H. Chen*, Academia Sinica, Taiwan, *L.C. Chen*, National Taiwan University

Optical sensing of adenine and thymine nucleic acid species have been achieved at the femtomolar level using self assembled gold and silver nanoparticles coated silicon nanotips (SiNTs) arrays. The use of sub-10 nm metal particulates with optimum density and inter-particle distance ensures such high levels of sensitivity in surface enhanced Raman scattering experiments. In this work wafer-scale silicon nanotip arrays were fabricated using a patented self masked dry etching technique to provide an excellent platform for the metal self assembly. This structure consists of the SiNTs with apex and bottom diameter of ~ 1 nm and ~ 100 nm, respectively, length of ~ 1000 nm and density of $10^{11}/cm^2$. The high density of gold and silver nanoparticles and short inter-particle distance enabled the bio-immobilization and amplification of the Raman signals of adsorbed molecules, allowing identification of minute amount of the adsorbed molecules with chemical specificity. The high sensitivity of surface enhanced Raman scattering can be maintained over a considerable period of time. The vibrational Raman signals of immobilized species can be detected even after four months of conservation. The straightforward, binder-less, stable and room temperature bio-molecular detection underlines the effectiveness of surface enhanced Raman scattering vis-á-vis fluorescence.

BI-ThP3 Characterization of Functionalized Layers on Silica Surfaces for DNA Attachment, ***R.A. Shircliff***, *J.F. Fennell*, Colorado School of Mines, *I.T. Martin*, *P. Stradins*, National Renewable Energy Laboratory, *S.G. Boyes*, Colorado School of Mines, *M.L. Ghirardi*, National Renewable Energy Laboratory, *S.W. Cowley*, Colorado School of Mines, *H.M. Branz*, National Renewable Energy Laboratory

The morphology and chemistry of functionalized silica surfaces have been characterized to understand key factors to surface uniformity and reproducibility of DNA immobilization and hybridization. Deposited 3-aminopropyltriethoxysilane (APTES) and 3-aminopropyldimethylethoxysilane (APDMES) layers were characterized by x-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), spectroscopic ellipsometry, thermogravimetric analysis, contact angle and DNA bioassays. DNA bioassays included fluorescence-based analysis and ^{32}P-radiometric assays of DNA surface density. Angle-resolved XPS confirmed attachment of the sulfo-EMCS heterobifunctional crosslinker to amine-terminated layers deposited from both APTES and APDMES. High levels of immobilization of thiolated single-strand DNA to APTES-modified surfaces were observed by fluorescence from fluorescein dye attached to the DNA. Surprisingly, there was no detectable attachment of thiolated DNA to surfaces modified with monolayer films from APDMES. AFM of the APTES film revealed up to micron-scale island formations, which were likely caused by polymerization in the solution phase or on the surface. The APTES films also had significant variations of morphology under nominally identical deposition conditions, which may correlate with irreproducibility in DNA attachment. In contrast, the APDMES films had sub-nanometer surface roughness. Deposition of APTES, commonly used in DNA microarrays, showed high immobilization efficiency but lacked good reproducibility. APDMES films, which can only form a monolayer, showed reproducible monolayer films but lacked measurable DNA attachment. As an alternative to silane films, preliminary results will be reported on poly(ethylene glycol)-based films in order to improve reproducibility of DNA immobilization. We gratefully acknowledge the NREL Laboratory Directed Research and Development program for project funding. One of us (JFF) was supported by the U.S. Army.

BI-ThP4 PNA-PEG Modified Silicon Platforms as Functional Bio-Interfaces for Applications in DNA Microarrays and Biosensors, ***A. Cattani-Scholz***, *D. Pedone*, *F. Blobner*, *G. Abstreiter*, Technical University Munich, Germany, *J. Schwartz*, Princeton University, *M. Tornow*, Technical University Braunschweig, Germany, *L. Andruzzi*, Ludwig-Maximilians University Munich, Germany

Bio-functional interfaces on semiconductor materials enjoy increasing interest in basic and applied sciences because of the many possible applications of these structures in, e.g., proteomics, micro-array technology and biosensors. For DNA sensing applications single stranded DNA or peptide nucleic acid (PNA) is commonly covalently immobilized via a linker onto the surface which has been pre-modified with a thin organic film before. Here, high hybridization efficiency is generally strived for, together with a maximum suppression of unwanted, nonspecific interactions between target DNA and the sensor surface. We report on the synthesis and characterization of two novel types of PNA interfaces on silicon/siliconoxide substrates featuring poly(ethyleneglycol) $(PEG)_n$ as molecular spacer and backfilling. As type one, phosphonate self-assembled monolayers were derivatized with a 12mer PNA oligomer via modification with and post-functionalization of a maleimide-terminated poly(ethyleneglycol) spacer (PEG_{45}). Similarly, a type two modification consisted of silane self-assembled monolayers which were functionalized with PNA via modification with a maleimide-terminated PEG_{45} spacer and were also subsequently modified with a shorter methoxy-terminated PEG_{12} (back-filling). X-ray photoelectron spectroscopy (XPS) analysis confirmed binding of PEG and PNA to the phosphonate and silane films and indicated that additional PEG chains were tethered to the surface during the backfilling process. We carried out hybridization experiments in the presence of matching and mismatching, fluorescently labeled DNA and found that both types of bio-functional surfaces were effective in the hybridization of matching DNA while significantly reducing non-specific adsorption. To verify the suppression of DNA adsorption on PEG-only modified surfaces and to extend the scheme towards laterally patterned structures we employed micro-molding techniques, i.e., pressed DNA-coated PDMS stamps onto a surface which comprised of alternating PNA functionalized, and non-functionalized regions, respectively, in a grid-like manner. These studies confirmed that hybridization took place selectively at the PNA functionalized regions only, while physisorption at the probe-less PEG-functionalized regions was drastically reduced.

BI-ThP5 Lipid Bilayer Formation and Properties Studied by Combined Electrical Impedance Spectroscopy and QCM-D, ***E. Briand***, *F. Höök*, *B. Kasemo*, *S. Petronis*, Chalmers University of Technology, Sweden

The added value of using synchronized Electrochemical Impedance Spectroscopy (EIS) and Quartz Crystal Microbalance with Dissipation (QCM-D) monitoring is that phenomena and properties, hidden for one of the techniques, may be dynamically resolved by the other one. EIS provides information about the electrical properties of the studied system, while QCM-D provides information about adsorbed mass variations and viscoelastic properties of the adlayer. We have here applied these combined techniques to study (i) supported lipid bilayer formation and (ii) subsequent pore formation using gramicidin D. The results demonstrate how these techniques in combination provide new insights about this and similar bio-adlayer systems. (i) The signatures, produced by the two techniques, of lipid bilayer formation on SiO2 from nanosized POPC liposomes, are quite different. The well established QCM-D signature tells that the initial kinetic phase consists of intact liposome adsorption, followed by vesicle rupture and fusion of lipid bilayer patches to a coherent bilayer. In contrast, EIS does not show any change in impedance until slightly before the critical liposome coverage is reached, where rupture and bilayer formation begins. Furthermore, at the end of the process, where the QCM-D Δf and ΔD signals have reached stable bilayer values, the electrical resistance still varies for several minutes, indicating a rearrangement/annealing process and/or additional minor addition of lipids. The absolute value of the bilayer resistance was found to significantly improve when cations were present in the buffer. (ii) Using a high resistance bilayer as the starting point, the insertion of gramicidin D was followed by QCM-D and EIS. By simultaneously recording EIS signals and changes in the viscoelastic properties (QCM-D) of the bilayer, at different GrD concentrations it was possible to identify the range of concentrations suitable for combined studies of the peptide activity and pore formation.

BI-ThP6 A Simple Method for Making Highly Ordered Chemical Patterns by Sputtering Through Ordered Binary Colloidal Crystals, *G. Singh, V. Gohri, S. Pillai, A. Arpanaei, M. Foss,* ***P. Kingshott***, The University of Aarhus, Denmark

Nanopatterning of biomolecules, such proteins, DNA, and polysaccharides are of great interest in cell culture dishes, biosensors, medical implants and tissue engineering. These so-called nanoarrays 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, or dip-pen AFM 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 polystyrene nanoparticle patterns can be generated from simple self-assembly onto surfaces, where single layers of large particles are surrounded by crystals of smaller particles. Here, we report a novel method for generating chemical nanopatterns by Au sputtering through the crystal layer followed by lift-off of the particles. The crystal regions of the binary pattern, composed of the smaller particles, facilitate transport of the Au sputter beam to the substrate. After particle lift-off only the regions where the small particles have been in contact with the silicon substrate are coated with Au. The large particles act as a mask and remain uncoated, and the thickness of the surrounding Au layer is controlled by the sputter time. The highly ordered chemical patterns are generated where the size of the features are tuned by appropriate choice of particle sizes (50nm to 3µm diameters) and ratios. The stability of the Au layers to aqueous environments is ensured by coating the Si wafer with a thiolated silane, which acts as an adhesion layer. 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 on to the Si regions of the pattern. The Au patterns and subsequent protein adsorption are characterized by AFM, SEM and fluorescent microscopy. XPS and ToF-SIMS are used to characterise the chemical modification steps of the patterning. In summary, we introduce a novel method for generating highly-ordered chemical nanopatterns that is very fast, inexpensive, and allows patterns of biomolecules to be created over large areas.

BI-ThP7 Smooth SiO_2 Surface for Biointerfaces Applications Obtained by Oxidation of Polysiloxane Thin Films, ***C. Satriano***, *G.M.L. Messina,* University of Catania, Italy, *S. Svedhem,* Chalmers University of Technology, Sweden, *G. Marletta,* University of Catania, Italy, *B. Kasemo*, Chalmers University of Technology, Sweden

A simple approach for preparation of smooth SiO_2 surfaces by oxidative modification of polysiloxane films is described. Thin films of poly(hydroxymethylsiloxane) (PHMS) were deposited by spin coating on silicon or gold substrates and modified either by radiofrequency oxygen plasmas or combined oxygen plasmas and thermal treatments. The modified films were converted to SiO_x phases, ultimately SiO_2 like, as determined by XPS, and exhibited very high water wettability, as measured by contact angle measurements. Moreover, the high original flatness of the PHMS was not affected by the modification treatments. Both untreated and treated films had roughness values below one nanometer. Using the QCM-D and SPR techniques the adsorption behaviour of and supported lipid membrane (SLB) formation, from small unilamellar vesicles of neutral zwitterionic POPC, positively charged DOEPC and negatively charged DOPC/DOPS mixtures were investigated onto untreated and modified PHMS films. SLBs were successfully obtained on the modified PHMS surfaces. The latter results are compared with corresponding results for PVD deposited SiO_2.

BI-ThP8 Influence of Raft Forming Lipids on Bilayer Formation on SiO_2 Surfaces Studied by Means of QCM-D, ***M. Sundh***, University of Aarhus, Denmark, *S. Svedhem, B. Kasemo,* Chalmers University of Technology, Sweden, *D. Sutherland*, University of Aarhus, Denmark

It is well known that artificial bilayers composed of ternary lipid mixtures of phosphatidylcholine, sphingomyelin (SM), and cholesterol (chol) phase separate and form domains enriched in SM and chol; so called rafts. Rafts are believed to be involved in numerous cellular processes such as cell signaling, endocytosis etc.[1] and thus the importance of their study. Developments in the field of nanotechnology have opened up new routes to the study of molecular systems. The long term goal of these studies involves the use of nanostructured interfaces to systematically define parameters such as membrane curvature and allow the investigation of their correlation to phase separation. As a preliminary step the formation and quality of bilayers, formed with lipid raft compositions, on flat surfaces is investigated. Quartz crystal microbalance with dissipation (QCM-D) is a tool commonly used to study and quantify the adsorption of proteins and the fusion of lipid vesicles into lipid bilayers.[2] In this study the influence of lipid composition in lipid vesicles on the formation of bilayers was investigated and interpreted in terms of the phase separation of the components. Ternary lipid mixtures of POPC/SM/chol at different ratios were formed into unilamellar vesicles by extrusion and deposited on SiO_2 coated QCM crystals. Preliminary results show that the formation of lipid bilayers can be tuned by changing the lipid composition or temperature. An increase in the proportion of SM within the vesicles results in a reduction in quality of the formed bilayer, seen by an increased dissipation response. These results can be interpreted in terms of phase separation into ordered and disordered fluid domains within the vesicles. As the SM concentration is increased the ordered phase becomes dominant up to a point where the vesicles are too rigid to fuse and form bilayers. A temperature study of vesicles with POPC/chol shows that rupture of vesicles could be induced by doing the experiment at increased temperatures and hence changing the lipid phase.

[1] Simons, K. and D. Toomre, Lipid rafts and signal transduction. Nat Rev Mol Cell Biol, 2000. 1(1): p. 31-39.

[2] Reimhult, E., F. Hook, and B. Kasemo, Intact vesicle adsorption and supported biomembrane formation from vesicles in solution: Influence of surface chemistry, vesicle size, temperature, and osmotic pressure. Langmuir, 2003. 19(5): p. 1681-1691.

BI-ThP9 Investigation and Reduction of Current Noise in Solid-State Nanopores, ***D. Pedone***, *M. Firnkes, G. Abstreiter, U. Rant*, Technical University Munich, Germany

Solid state nanopores have emerged as powerful analytical tools to study single molecules. DNA translocation experiments have been conducted with great success in the past, and protein translocation has been demonstrated recently. In these experiments, the biomolecule is detected by measuring the ionic current through the pore, which becomes transiently suppressed when a molecule traverses the pore. The use of solid-state nanopores for studies of complex biomolecular behavior or interactions relies on the ability to record these current blockades with superior fidelity, which poses great challenges with respect to the noise characteristics of the solid-state device. Here we present systematic studies addressing the current noise of solid-state nanopores for translocation experiments. Pores with diameters <10 nm have been fabricated in Si_3N_4 membranes by e-beam lithography and subsequent shrinking in a transmission electron microscope. The electrical characteristics of the nanopore chips in aqueous pH-buffered saline solutions are studied using electrochemical impedance spectroscopy (EIS), cyclic voltammetry, and potential step methods. Equivalent circuit models to represent the nanopore device are proposed based on the obtained data. The frequency dependence of the current noise is recorded with spectral analyzers and discussed within the extracted equivalent circuit models. Within this framework, we investigate the influence of various parameters on the electrical noise: (i) chip designs with different membrane dimensions are realized by combining optical and e-beam lithography with feedback-etching methods, (ii) surface passivation using silicone elastomers and photo-resists are compared, (iii) the composition, salinity, and pH value of the buffer solution is examined. Our results allow us to identify the contribution of various capacitances and dielectric losses across the chip to the measurement noise and suggest guidelines for low-noise translocation recordings.

BI-ThP10 Fabrication and Chemical Surface Treatment of Solid State Nanopores in SiN Membranes, ***M. Firnkes***, *D. Pedone, G. Abstreiter, U. Rant*, Technical University Munich, Germany

Solid state nanopores attracted broad attention in recent years as a tool to study biological molecules like DNA or proteins. In these experiments, the translocation of the molecule through the nanopore is detected by a blockade of the ionic current across the pore. Up to now solid state nanopores are mainly directly drilled into a freestanding silicon nitride membrane via an intense e-beam. Here we report a new pore fabrication technique. Single nanopores are processed in silicon nitride membranes by e-beam lithography and feedback-controlled wet chemical etching, followed by TEM induced shrinking. Moreover we present current noise data showing the influence of various chemical treatments of the pore surface. Starting with a (100) silicon chip of 200 µm thickness, which features 50 nm silicon nitride coatings on both sides, we use optical lithography to form an etch mask on the chip's back side for anisotropic etching of the silicon with KOH. Subsequently we utilize e-beam lithography on the front side to open holes of 40 – 50 nm in the silicon nitride. In the next step the silicon is etched by KOH resulting in a pyramidal shaped undercut of the small holes on the chip front side. During a second KOH etching process from the backside only, we observe the time dependence of the electrical current across the silicon chip. The etching is stopped when a certain current threshold indicates the opening of the pyramid. In this way the pyramid is truncated in a controlled manner. This leads to a 5 x 5 µm freestanding silicon nitride membrane containing the pore. To get the desired pore size we shrink the pores using a TEM. Electrical noise analysis data is presented showing the influence of small membrane sizes resulting from feedback-controlled etching. In addition we studied the influence of the surface termination on wetting properties and electrical noise. In this context we applied both oxidizing (HF) as well as reducing agents (piranha, oxygen

plasma) to change the surface properties of the nanopores. Our results show the benefits of the combination of feedback chemical etching and standard nanopatterning techniques on the electrical noise and indicate how current recordings can be obtained with low noise by a chemical treatment of the nanopore.

BI-ThP11 Determination of Protein Charge with Switch DNA Bio-Sensors, ***J. Knezevic***, *W. Kaiser, E. Pringsheim, G. Abstreiter, U. Rant*, Technische Universität München, Germany

In the recent past, switchable DNA layers have been established as promising candidates for biosensors.[1] Here, the efficiency and dynamics of the electrically induced conformation-switching of surface tethered DNA molecules are used as the sensing parameters. The detection of DNA and proteins (antibodies and antibody fragments) has been demonstrated. Moreover, the size of the captured protein targets could be determined from the switching dynamics on-chip. However, the influence of the proteins' charge remained unclear in these experiments. Here we report on DNA-switching bio-sensing experiments, where the influence of the protein charge was investigated on the basis of the avidin/ streptavidin/ neutravidin-model system. The modulation amplitude of the switching DNA layer was probed electro-optically at low frequencies of the driving electrical signal. The switching kinetics of the tethered molecules were analyzed by frequency resolved measurements. In addition, the double layer charging process was evaluated by impedance measurements. The proteins' charge was varied on-chip by altering the solution pH value. In complementary measurements, the target proteins were characterized regarding their charge and size by dynamic-light scattering. The correlation between the protein charge and size and the low-frequency switching behavior is evaluated. Further, the influence of the protein charge and size on the switching dynamics is described. The results are discussed within the framework of classical electrostatic screening models. Finally, we elucidate the possibility to employ switchable DNA layers for the charge-sensitive detection and characterization of proteins, as well as biomolecules in general.

[1]Rant et al., PNAS 2007, vol. 104, pp. 17364-17369.

BI-ThP12 Surface-enhanced Raman Scattering from Controlled Nanoparticle System, ***S.Y. Chen***, *D.S. Sebba, A.A. Lazarides*, Duke University

We present a theoretical and experimental study of surface-enhanced Raman scattering from core-satellite nanoparticle assemblies of known structure in the solution phase. The detectability of the Raman signal is attributed to enhanced electromagnetic fields localized between plasmonically coupled core and satellite metal nanoparticles. Design of the structures was accomplished using near field calculations based upon Generalized Mie theory, a theory that accurately accounts for multipolar coupling within clusters of spherical particles. Assembly structures are identified on the basis of positions of hot spots and overlap of plasmon resonance frequencies with Raman excitation spectra and available laser lines. Core-satellite structures are assembled using DNA linkers and characterized by transmission electron microscopy (TEM). The control of field strength and plasmon frequency provided by the coupled particle system is expected to provide insight into SERS signaling of use in application of SERS to biomolecule sensing.

BI-ThP13 Magnetic Tweezer Sensor for Ensemble Binding Events of Nonmagnetic Particles, ***R.M. Erb***, *R.E. Ducker, S. Zauscher, B.B. Yellen*, Duke University

Current methods to measure molecular binding strengths include force pulling methods such as atomic force microscopy and optical tweezing. These methods are greatly limited in their throughput, requiring molecular fishing and individual particle targeting that produces binding data on the order of minutes or hours. To overcome these deficiencies, the authors have developed a magnetic system that allows for fast ensemble measurements of thousands of single particle-substrate bonds simultaneously using a High Gradient Magnetic Separation (HGMS) system. The described system is a dense array of micron-size ferromagnetic thin islands on a substrate. These magnetic arrays offer the ability to apply very strong particle forces that can be in the range of tens of nanonewtons, orders of magnitude higher than most optical or electrical systems. Additionally, this system can be used to apply forces on nonmagnetic particles by submerging them in biocompatible magnetic fluids, a technique known as "inverse" magnetophoresis. The ferrofluid causes the nonmagnetic particles to behave as magnetic holes allowing for particle-substrate bonds to be broken through the islands' applied magnetic force. The authors have extended this system onto the surface of a quartz crystal microbalance (QCM) sensor, which allows for the accurate sensing of ensemble particle movement. To test this system, the authors use a mixed self-assembled monolayer of biotin and oligoethylene glycol and selectively bind streptavidin coated particles to the magnetic islands. Using the magnetic islands and an external magnetic field, streptavidin particles are attracted to a preprogrammed edge of the islands and are allowed to undergo molecular binding with the surface. As an opposite external field is applied, the particles will be pulled en masse to the opposite side of the islands, a movement that can be sensed and analyzed by the QCM. Through knowing the applied magnetic forces, this system allows for the ensemble quantification of bond dissociation between any chemically active particle and substrate.

BI-ThP14 Nanoparticle Thermoplasmonic Modulation, *R.H. Farahi,* ***A. Passian***, *A.L. Lereu, T.G. Thundat,* Oak Ridge National Laboratory, *Y. Jones*, Alcorn State University

Single particle thermo-optical properties are increasingly important in applications such as therapeutics, nano devices, and alternative energy sources. In these applications, the temperature dependent electronic characteristics play a role in the feasibility, efficiency, and the functionality of the intended system. We present an investigation of the thermal properties of gold nanoparticles on a quartz substrate using optical excitation of surface plasmons. The surface deformation, in the region of the localized optical modulation of the surface plasmons, is studied as a function of frequency, power, and polarization. A non-linear frequency and power dependence is observed for the nanostructure system as a result of the thermoplasmonic processes including the volumetric deformations. A threshold power for the observation of the modulation is estimated and is in good agreement with theoretical and computational results.

BI-ThP15 Where DNA and Plasmonics Meet- An Investigation into Cooperative Molecular Recognition at a DNA Nanostructure-Metal Interface, ***E.R. Irish***, *T.H. LaBean, A.A. Lazarides*, Duke University

Recent work in assembly of complex DNA nanostructures has demonstrated the effectiveness with which the non-covalent forces of DNA hybridization can drive formation of a topologically rich set of engineered DNA nanostructures. These DNA nanostructures can be used as structural components within a variety of complex nanosystems, including integrated systems for molecular detection. With the advances in the design and solution phase assembly of novel addressable DNA nanostructures, there is a need for the development of new techniques for controlling deposition of the structures on surfaces. The objective of this research is to investigate thermodynamic and kinetic control of interactions between DNA nanostructures and oligonucleotide functionalized gold films. In this research, surface plasmon resonance (SPR) is used for real-time monitoring of the hybridization of DNA structures on oligonucleotide functionalized gold films. Kinetic and thermodynamic parameters derived from the SPR reflectivity data are used to evaluate the effect of multivalence on the strength of interaction. Kinetic measurements, such as the association and dissociation rates, are determined through the monitoring of the SPR response to hybridization as a function of concentration. Ultimately, understanding of the kinetic and thermodynamic parameters that characterize multivalent interactions between DNA nanostructures and gold films will enable engineering of interactions at soft/hard matter interfaces. It is anticipated that the new tools for integrating soft matter on patterned templates will prove useful in future applications of DNA nanostructures that require organization of the soft matter.

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Shohet, J.L.: BI-TuM12, 4
Shumaker-Parry, J.S.: BI+TF+MI+NS+NC-ThA3, **26**
Simnick, A.J.: BI-TuP5, 11
Singh, G.: BI+NC-TuA3, **7**; BI-ThP6, 31
Sinner, E.-K.: BI+SS+NC-MoA5, 1; BI+SS+NC-MoA7, **1**
Sirghi, L.: BO+EM+BI+NC-ThM6, 24; BO+NS+BI+NC-ThA11, **29**
Sjovall, P.: BO+AS+BI-WeA10, **21**
Sjövall, P.: BI+NC-ThM4, 22; BO+AS+BI-WeA12, 21
Skeldal, S.: BI+NC-TuA8, 8
Skuza, J.R.: BI+TF+MI+NS+NC-ThA7, 26
Smith, A.M.: BI+NC-ThM1, 22
Smith, D.R.: BI+TF+MI+NS+NC-ThA10, 27
Snijders, J.H.M.: BI-TuP11, 12
Söderlind, F.: BO+EM+BI+NC-ThM5, 24
Spatz, J.P.: BO+NS+BI+NC-ThA3, 28
Spycher, P.: BI+NC-TuA5, 7
Stayton, P.S.: BI-TuM9, 4

Steinem, C.: BI+NC-ThM9, **23**
Steitz, R.: BO+AS+BI+NC-WeM2, **16**
Storts, D.: BI+NC-TuA9, 8
Stradins, P.: BI-ThP3, 30
Stuart, S.J.: BI-TuM3, 3; BI-WeA5, 18
Styer, A.L.: BI+NC-TuA12, 8; BI-TuP6, 12
Sullivan, S.P.: BI+NC-TuA12, 8; BI+NC-TuA4, **7**
Sundh, M.: BI-ThP8, **31**
Sutherland, D.: BI+NC-ThM5, **22**; BI-ThP8, 31
Svedhem, S.: BI+NC-ThM4, **22**; BI-ThP7, 31; BI-ThP8, 31; BI-WeA4, 18; BO+AS+BI-WeA10, 21
Szymonski, M.: BO+EM+BI+NC-ThM11, **24**

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Tabari, A.M.: BI+NC-ThM11, 23
Takai, O.: BI-TuP17, 14; BI-TuP18, 14
Takei, H.: BO+NS+BI+NC-ThA2, **27**
Tan, S.: BI+SS+NC-MoA8, 1
Tatematsu, H.: BI-TuP18, **14**
Taylor, M.: BO+PS+AS+BI+SS-TuA1, 9
Tegenfeldt, J.: BI+NC-WeM11, 16
Tekiel, A.: BO+EM+BI+NC-ThM11, 24
Teplyakov, A.V.: BI+NC-TuA4, 7; BO+EM+BI+NC-ThM9, 24
Terfort, A.: BO+AS+BI+NC-WeM1, 16
Textor, M.: BI+NC-ThM11, 23; BI+NC-TuA5, 7; BO+NS+BI+NC-ThA8, 28
Theilacker, W.M.: BI+NC-TuA12, 8; BI-TuP6, **12**
Thissen, H.: BI-TuM11, 4
Thomas, H.: BO+AS+BI+NC-WeM1, 16
Thundat, T.G.: BI-ThP14, 32
Ting, Y.H.: BO+PS+AS+BI+SS-TuA8, 9
Tomanek, D.: BO+EM+BI+NC-ThM12, 25
Tornow, M.: BI-ThP4, 30
Tosatti, S.: BO+NS+BI+NC-ThA8, 28
Tsang, K.: BI-TuM11, 4
Twiss, J.L.: BI+NC-TuA12, 8; BI-TuP6, 12

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Urquhart, A.J.: BO+PS+AS+BI+SS-TuA1, 9
Uvdal, K.: BO+EM+BI+NC-ThM5, 24

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Vahlberg, C.: BO+EM+BI+NC-ThM5, 24
Valsesia, A.: BI+TF+MI+NS+NC-ThA5, 26; BO+NS+BI+NC-ThA5, 28; BO+NS+BI+NC-ThA9, 28; BO+PS+AS+BI+SS-TuA11, 10
van der Horst, M.A.: BI-TuP16, 13
Van der Marel, C.: BI-TuP11, **12**
Van Oers, D.D.C.A.: BI-TuP11, 12
Vasudevan, S.: BI+NC-WeM3, **15**
Vellore, N.: BI-WeA5, **18**
Vogler, E.A.: BI-TuP1, 11

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Wahl, K.J.: BO+AS+BI-WeA2, 20
Walczak, K.: BI+NC-WeM3, 15
Walker, A.V.: BO+AS+BI-WeA9, **20**
Walt, D.R.: BI+NC-WeM12, 16
Wang, G.: BI-WeA4, 18
Wang, H.T.: BO+EM+BI+NC-ThM1, 23
Wang, P.: BO+AS+BI+NC-WeM11, 17
Wang, Y.L.: BO+EM+BI+NC-ThM1, 23
Wei, Y.: BI-TuP20, 14
Weidner, T.: BI-WeA12, **19**; BO+EM+BI+NC-ThM10, 24
Wendt, A.E.: BO+PS+AS+BI+SS-TuA8, **9**
White, R.G.: BI-WeA3, 18
Whitman, L.J.: BI-TuM10, 4
Whitty, A.: BI+TF+MI+NS+NC-ThA4, 26
Wieland, M.: BI-TuP12, 13
Willis, D.E.: BI+NC-TuA12, 8; BI-TuP6, 12
Wind, S.J.: BO+NS+BI+NC-ThA1, 27
Winkler, T.: BO+AS+BI+NC-WeM1, 16
Wright, P.C.: BO+NS+BI+NC-ThA10, 29; BO+PS+AS+BI+SS-TuA5, 9
Wurpel, G.W.H.: BI-TuP16, 13

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Yakimova, R.: BO+EM+BI+NC-ThM5, 24
Yamada, T.: BI-TuP16, 13
Yang, K.: BI+TF+MI+NS+NC-ThA7, 26
Yang, T.: BO+EM+BI+NC-ThM12, 25
Yasuda, K.: BO+NS+BI+NC-ThA2, 27
Yellen, B.B.: BI+NC-TuA11, 8; BI-ThP13, 32
Yoshida, K.: BI+NC-TuA8, 8

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Zachar, V.: BI+NC-TuA8, 8
Zangmeister, R.A.: BI-TuP7, **12**
Zauscher, S.: BI+NC-TuA11, 8; BI-ThP13, 32; BI-TuP5, 11
Zhang, C.: BI+NC-ThM6, 22; BI-TuM6, **3**
Zhang, L.: SY+SS+BI-TuM12, 5
Zhang, W.H.: SY+SS+BI-TuM12, 5
Zhang, X.: BI+NC-TuA4, 7; BI+TF+MI+NS+NC-ThA1, **26**; BO+EM+BI+NC-ThM9, **24**
Zhang, Z.: BI+NC-TuA12, 8
Zhao, K.: BI+NC-TuA9, 8
Zhao, W.: SY+SS+BI-TuM12, 5
Zharnikov, M.: BO+AS+BI+NC-WeM1, 16; SY+SS+BI-TuM5, **5**
Zhou, C.: BO+AS+BI-WeA9, 20
Zhu, J.F.: SY+SS+BI-TuM12, **5**
Zhu, X.-Y.: BI+NC-ThM3, **22**
Zuercher, S.: BO+NS+BI+NC-ThA8, 28