

Biorganic functionalization of gold thin films and nanocluster mesoscalar substrates including Si, SiC, GaN and GaAs. The advantages of this system nanoparticles (NPs) self-assembled on a large variety of semiconductor surfaces including Si, SiC, GaN and GaAs.

In this presentation we will discuss bio- and chemofunctionalization of a novel system based on plasmonic gallium (Ga) nanoparticles (NPs) self-assembled on a large variety of semiconductor substrates including Si, SiC, GaN and GaAs. The advantages of this system nanoparticles (NPs) self-assembled on a large variety of semiconductor substrates including Si, SiC, GaN and GaAs.

For both systems Ga (NPs)/semiconductor and Au(NPs)/semiconductor we discuss semiconductor surface treatments affecting the interface chemistry and the dynamics of interface phenomena playing a role in tailoring the surface plasmon resonance. Those systems have also been functionalized by bio-molecules, e.g., antigens and antibodies for biosensing and by porphyrins for gas sensing to nitric oxide (NO).

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

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

Data are corroborated by atomic force and electrical force microscopies.

This work is supported by the TFP European Project NanoCharM Multifunctional NanoMaterial Characterization exploiting ellipsometry and polarimetry

BI+NS-MoM2  Geometry and Interactions: How Shape and Intermolecular Interactions Direct the Self-Assembly of Cage Molecules on Au(111).


The self-assembly of cage molecules on metal surfaces enables the manipulation of interface chemistry while eliminating an entire class of defect modes commonly associated with straight-chain molecules such as the n-alkanethiols. The adamantanethiols and carbonanethiols have similar geometries and unit cells on Au(111), but exhibit striking differences in their behavior. We attribute these differences to their contrasting electronic structure and consequent intermolecular interactions in domains of random shape and size.

We have characterized the SAMs of positional isomers of several carbonanethiols and adamantanethiols by scanning tunneling microscopy, cyclic voltammetry, grazing-incidence Fourier transform infrared spectroscopy, Kelvin probe microscopy, and contact-angle goniometry. We discuss how geometry and intermolecular interactions play competing roles in determining monolayer assembly and stability.

BI+NS-MoM3  On the Role of Supramolecular Nanostructure in determining Interfacial Energy and Biological Interactions.

F. Stiefacl, Massachusetts Institute of Technology

We present a new nanoparticle placement technique in which single nanoparticles of different sizes recognize different target positions on a substrate. By placing only one nanoparticle in each target position in a self-limiting way, we demonstrate this by using ~50nm and ~20nm colloidal Au nanoparticles (AuNPs) as a model system, where the ~50nm particles are firstly electrostatically guided onto targeted substrate locations and then the ~20nm particles to different target locations on the surface. The displacement of CTAB allows functionalization with other molecules phase-separate in domains of random shape and size. Very often SAMs composed of more than one type of molecule (mixed-SAMs) are used to impart multiple properties. Scanning tunneling microscopy (STM) studies have shown that, in mixed SAMs, molecules phase-separate in domains of random shape and size.

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

BI+NS-MoM5  Can We Make Alkanethiol SAM's on Surface of Gold Nanorods?

S. Chakraborty, S. Lee, V. Perez-Luna, Illinois Institute of Technology

Gold has been a fascinating element that drew the interest of people since early origins of human history. However, it is just over the last two decades that it gained a resurgence of interest, in conjunction with the advent and development of nanotechnology. Gold nanoparticles have a wide variety of bio-applications such as labeling, sensing, and targeted drug delivery. A directed assembly of gold nanoparticles in the form of nanowires or networks is extensively used as nanodevices, mostly comprising of a variety of shapes; spheres, rods and cubes being the most commonly observed shapes. The peculiarity of our study is the exploitation of Plasmonic spectroscopic ellipsometry (HORIBA UVISEL, Jobin Yvon) for the optical monitoring of the plasmon resonance tuning in real-time during the nature of the deposition on semiconductor surfaces and for the functionalization of metal nanoparticles by anchoring biomolecules to the semiconductor nanostructures.

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

Data are corroborated by atomic force and electrical force microscopies.

This work is supported by the TFP European Project NanoCharM Multifunctional NanoMaterial Characterization exploiting ellipsometry and polarimetry.

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

We present a new nanoparticle placement technique in which single nanoparticles of different sizes recognize different target positions on a substrate. By placing only one nanoparticle in each target position in a self-limiting way, we demonstrate this by using ~50nm and ~20nm colloidal Au nanoparticles (AuNPs) as a model system, where the ~50nm particles are firstly electrostatically guided onto targeted substrate locations and then the ~20nm particles to different target locations on the surface. The displacement of CTAB allows functionalization with other molecules phase-separate in domains of random shape and size. Very often SAMs composed of more than one type of molecule (mixed-SAMs) are used to impart multiple properties. Scanning tunneling microscopy (STM) studies have shown that, in mixed SAMs, molecules phase-separate in domains of random shape and size.

We will show that when mixed SAMs are formed on surfaces with a radius of curvature smaller than 20 nm they spontaneously phase-separate in highly ordered phases of unprecedented size. The reason for this supramolecular phenomenon is purely topological and can be rationalized through the “hairy ball theorem”. In the specific case of mixed SAMs formed on the surface of gold nanoparticles, the molecular ligands separate into 5 Å wide phases of alternating composition that encircle or spiral around the particle metallic core. This new family of nano-structured nanomaterials shows new properties solely due to this novel and unique morphology. For example, we will show that the cell uptake of these particles strongly depends on the particle’s composition and the ligand shell morphology.
same substrate. The electrostatic guiding structure was defined using CMOS-compatible fabrication processes and subsequent functionalization of surfaces using self-assembled monolayers (SAMs) of organofluorine compounds. Using appropriate guiding structure, we present >90% success rate of ∼50nm AuNPs placement onto substrate locations targeted for ∼50nm AuNPs only. Theoretical calculations for ∼50nm AuNPs, which was carried out by solving the non-linear Poisson-Boltzmann equation, revealed that the self-limiting single-particle placement is due to an increase of the free energy barrier after placement of one nanoparticle onto a targeted substrate location, which prevents the approach of other nanoparticles to the already occupied position. The size-selective placement of single nanoparticles can be explained by dependence of the free energy barrier changes upon the sizes of nanoparticles and guiding structures. The same approach may also be useful for size-selective and single-entity-level placement of other nanoscale building blocks such as nanowires, proteins, and DNA. Supported by NSF CAREER (ECS-0449958), ONR (N00014-05-1-0030), and THECB (003656-0014-2006)

**Nanometer-scale Science and Technology**

Room: L - Session NS+Bi-MoM

**Nanowires and Nanoparticles I**

Moderator: L.E. Ocola, Argonne National Laboratory

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

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

8:40am **NS+Bi-MoM2** Formation of Metallic Nanoclusters on TiO$_2$ by Deposition and Sputtering, A.B. Arjad, J.A. Yarmoff, University of California, Riverside

The discovery that Au nanoclusters are catalytically active when supported in an oxide matrix has led to an abundance of surface studies, particularly those concentrating on metal nanoclusters deposited on single crystal TiO$_2$. For example, STM showed that Au clusters grow on TiO$_2$(110) without a wetting layer and that the size of the clusters increases as more metal is deposited. We previously used low energy alkali ion scattering to interrogate such clusters grown by evaporation, measuring the neutral fraction of the backscattered ions with time-of-flight spectroscopy. We were able to show that the neutralization depends on the electronic structure of the clusters, and therefore changes as a function of cluster size. Our group also demonstrated a novel method for the production of nanoclusters by Ar$^+$ sputtering a thin Au film deposited on TiO$_2$. As this initial work was performed with Au, it is not clear whether nanoclusters displaying quantum size effects could be fabricated by sputtering other materials. In the work presented here, Ag and Pt are evaporated onto TiO$_2$(110) and thin films of these metals are sputtered. The materials are then interrogated via the scattering of 2.5 keV Na$^+$. It was found that while both Pt and Ag form nanoclusters by evaporation, only Pt forms nanoclusters after sputtering. This may be due to differences in the surface free energy of the metals, or of the propensity of Ag to oxidize. Future efforts will include temperature programmed desorption to test the chemical activity of the metal nanoclusters, as well as the use of other substrates.

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

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

Iron (Fe) oxides and hydroxides have been the subject of numerous investigations for many years. Fe oxides and hydroxides find application in chemical catalysis, magneto-electronics, energy storage and conversion, and nuclear industry. The properties and phenomena of these materials are influenced by the morphology and size. The present work is performed to grow high-quality and ordered structure Fe oxides by simple wet-chemical method at a relatively low temperature. Fe oxides and hydroxides have been prepared in aqueous media by precipitation of iron nitrate and ammonium hydroxide. Their growth and structural characteristics have been investigated using x-ray diffraction (XRD) and high-resolution scanning electron microscopy (HRSEM).

The approach is preparing different solutions of Fe particles in suspension and acetic acid at different concentrations and subject to heat treatment at 100 °C. XRD analyses indicate that the solids obtained from aqueous stage exhibit goethite (α-FeO(OH)) with average particle size 2-3 nm, while the samples with acetic acid addition shown hematite (α-Fe2O3) with average size ~20 nm. HRSEM images confirm the presence of particles ~100 nm in samples with no acetic acid. HRSEM images confirm that the samples with acetic acid exhibit nanoparticles ~20 nm with small spots within the particles. In addition, SEM shows the presence of net-shaped structures and particles with high-density porosity within the nano-regime depending on the concentration of acetic acid. The experiments also confirm that Fe oxide nano-particles and networks are stable to a temperature of 500 °C. The results obtained on the growth, structure, and properties of the grown Fe-based nanomaterials will be presented and discussed in detail.

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

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

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

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


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

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


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

Monday Morning, November 9, 2009
Biomaterial Interfaces
Room: K - Session BI-MoA

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

2:00pm BI-MoA1 Bioengineering Stem Cell Fate, H.M. Blau, K. Havenstrite, Stanford University INVITED
A major challenge facing stem cell biologists is an understanding of the mechanisms that direct stem cell fate: the delicate balance between quiescence, self-renewal, and differentiation. Adult stem cells are localized in niches, specialized microenvironments, which protect them from differentiation. Upon culture, adult stem cells lose their “stemness,” or ability to self-renew. We have engineered artificial in vitro microenvironments that mimic key biochemical characteristics of adult stem cell niches in order to analyze the properties of stem cells and influence their fate. Microwell arrays are produced as a topographically structured polymer hydrogel surfaces allowing exposure of single cells either to soluble or tethered niche proteins. Using this platform, phenotypic and dynamic analyses of thousands of individual cells can be monitored simultaneously by time lapse microscopy. We have found that single proteins alter proliferation kinetics and asymmetric division behavior, leading to muscle and hematopoietic stem cell self-renewal in culture. Our data demonstrate that parameters of proliferation behavior in vitro correlate with stem cell function assayed in vivo. Ultimately, the goal of these studies is to increase our understanding of stem cell biology, expand stem cells in vitro for clinical applications, and discover new drugs for stimulating a patient’s own stem cells.

2:40pm BI-MoA3 An Investigation of Human Embryonic Stem Cell Attachment on 496 Different Acrylate Polymers in a Microarray: The Importance of Surface Chemistry as Probed by ToF SIMS, M.R. Alexander, J. Yang, M.C. Davies, The University of Nottingham, UK, Y. Mei, D.G. Anderson, R.S. Langer, MIT, M. Taylor, A.J. Urquhart, The University of Nottingham, UK
The relationship between the surface chemistry of materials and human cellular response has great importance in existing and emerging technology areas such as tissue engineering, regenerative medicine and biosensors. Here, we investigate hESC attachment, surface chemistry (using time of flight secondary ion mass spectrometry (ToF SIMS) and XPS) and bulk properties (using confocal Raman spectroscopy) of a large set of samples with diverse chemistry. These are acrylate polymers in the form of microspots in an array made from 22 different acrylate monomers mixed pairwise in different proportions and UV photopolymerised to give 496 unique micro- and co-polymers. We do not find a correlation between the human embryonic stem (hES) cell number and wettability, or surface elemental or functional composition that holds for all the samples on the array. In contrast, surface mass spectrometric data acquired using ToF SIMS correlate strongly with cell attachment on all polymers using partial least squares (PLS) regression. The ability to predict cell attachment using the SIMS data indicated that it contains sufficient information on the surface chemistry of the polymers to describe the effect of surface chemistry on cell attachment. Some of the moieties identified using this approach are consistent with previous theories relating surface chemistry on protein adsorption and in turn to cell adhesion, whereas others are new.

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

3:40pm BI-MoA6 Design of Protein Polymers as Novel Tissue Engineering Scaffolds, D. Sengupta, S.C. Heilshorn, Stanford University
The ability to tailor specific cell-matrix interactions in biomaterials is now recognized as an important method to control cell behaviour. Biomaterial adhesivity and elasticity are important determinants of cell adhesion, proliferation, and differentiation; and a coordinated cell response to these different material inputs results in complex signaling crosstalk. Independent modification of these biomaterial properties is thus extremely important, but difficult to achieve with current synthetic as well as natural biomaterials. While natural biomaterials such as collagen and Matrigel do not allow for independent tuning of multiple biomaterial properties, synthetic biomaterials such as PEG and acrylates can be toxic and immunogenic. An alternative approach to natural as well as synthetic materials is the use of protein polymers made with recombinant protein engineering technology. By templating protein synthesis using the genetic code, we have exact molecular-level control over our material. Using this strategy, we have engineered a family of tunable and biodegradable protein-engineered biomaterials that manipulate critical elements of the natural extracellular matrix. The materials are manufactured using a modular design strategy, resulting in a fusion protein comprised of multiple peptide domains that provide cell adhesion and matrix elasticity. Specifically, the elastic modulus of the material can be tuned (from ~3-1200 kPa) independently of RGDF ligand density (from 0-9300 cell adhesion sites), enabling optimization of the biomaterial interface for specific tissue engineering applications. Additionally, these interfaces can be easily micro-molded to incorporate micro- or nanoscale topographical features that induce cell alignment. Human embryonic stem cell-derived cardiomyocytes as well as mouse embryonic stem cells cultured on our protein-engineered biomaterials demonstrate viability, proliferation, differentiation, and morphology comparable to positive gelatin controls, providing a viable alternative to commonly used materials. The surface-level design strategy of these protein polymers allows for unprecedented control over the biomaterial-cell interface for regenerative medicine applications.

Monday Afternoon, November 9, 2009
4:00pm BI-MoA7 Axon Guidance on Patterned Gradients of Extracellular Matrix Proteins, W. Thielacker, H. Bui, University of Delaware, S. Sullivan, Alcoa Technical Center, L. Capriotti, University of Delaware, D. Willis, J. Twiss, Alfred I. duPont Hospital for Children, N. Zander, Army Aberdeen Research Laboratory, Z. Zhang, Excellatron, T. Beebe Jr, University of Delaware
This presentation will focus on axonal extension experiments made possible by recent developments in a general platform for substrate patterning of protein and peptide gradients using covalent attachment schemes, and employing cell- and protein-resistant lanes of PEO-like comb polymer. The platform allows step gradients in local and continuous gradients in local and continuous and peptide concentrations from micron to centimeter length scales. Control of the local surface density of proteins and peptides allows cell culture assays involving competition of cells for different extracellular matrix (ECM) proteins, propensity of axons to cross from one ECM protein lane into a different ECM protein lane, neuron attachment propensity, axon extension direction and rate, and controlled studies of cell-cell interactions between different cell types. This presentation will address the relationship between the local protein coverage and the “bioactivity” or “bioavailability,” using a variety of surface analytical techniques including XPS, TOF-SIMS and AFM, and optical microscopy techniques including epi- and confocal fluorescence microscopy.

Spinal cord injuries are one of the most catastrophic and costly types of injuries since damaged axons in the central nervous system are unable to spontaneously regenerate. Although reconstruction of damaged and diseased neural pathways remains a major hurdle, recent research has shown that aligned electrospun fiber mats can provide contact guidance cues for neurite outgrowth by acting as a bridging device. However, due to the nanometer sized fiber diameter and highly aligned nature of the scaffolds, the low interfiber distance limits penetration of the cells into the scaffold. To study the effect of fiber mat porosity on cellular infiltration, aligned fiber mats were fabricated via co-electrospinning poly(caprolactone) with polyethylene oxide (a water soluble polymer). Variation of the fabrication parameters allowed for control of the porosity of the scaffold with a full range of porosity (0-45%) through variation of the electrospinning parameters. As the surface composition is also critical in providing biochemical signals to direct neurite growth, the surfaces of the fibers were functionalized via air plasma treatment followed by attachment of several extracellular matrix proteins. The surface chemistry was characterized by X-ray Photoelectron Spectroscopy, Time of Flight Secondary Ion Mass Spectrometry, and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy. Cellular infiltration and neurite outgrowth of PC12 cells were evaluated for fiber mats of varying porosity and surface composition.
The mechanism of platelet-adhesion plays an important role in hemostasis and thrombosis. When blood vessels are injured or disrupted, the platelets membrane presented glycoprotein Ibα (GPIbα) will bind to von Willebrand Factor (vWF), which is a large multimeric plasma protein immobilized on collagen fibers. The binding interactions are regulated by the applied fluid shear rates. In this paper, we conducted a study of blood platelets adhesion on vWF A1 domain coated glass slides at different shear rates. The platelets movements are recorded by a high speed camera at 30fps. The recorded videos are analyzed using video/image processing software in order to calculate the platelet velocities at different shear rates as well as different protein coating concentrations. We found the average velocity decreased when the shear rate increased. This showed the binding requires high fluid shear rate in the flow. PLGA beads with the shapes of ellipsoid and sphere of about 1µm diameter were also studied using this method. However, the beads were coated with vWF A1 domain and the flow chambers were coated with GPIbα. We observed the decrease of the velocity with the increased shear rates, which showed increased binding strength between vWF A1 domain and GPIbα protein at higher shear rates. The ellipsoid beads had lower velocity comparing with the sphere beads at the same shear rate due to the larger contact area to the coating surface.

5:00pm BI-MoA10 Platelets Adhere to Adsorbed Albumin through a Receptor-Mediated Process, B. Sivaraman, R.A. Latour, Clemson University
Since albumin (Alb) lacks known sequences recognized by platelet receptors, it is not supposed to support platelet adhesion. However, studies have suggested that platelets may be able to adhere to adsorbed Alb (1, 2) with a mechanism related to adsorption-induced Alb unfolding (1), although the mechanisms of this remain unclear. To address this issue, we conducted studies to definitively determine if platelets adhere to adsorbed Alb, whether adhesion is related to adsorbed Alb conformation, and if it occurs by a receptor-mediated process. Alb was adsorbed at 0.1, 1.0, and 10 mg/mL on various alkanethiol SAM surfaces to vary the degree of unfolding in the adsorbed Alb. The adsorption-induced conformational changes in Alb was quantified by CD spectropolarimetry (3). Platelet adhesion studies were carried out and the platelet response determined by LDH assay and SEM. A series of platelet adhesion inhibitors and protein modification agents were used to probe the mechanisms of platelet adhesion. Platelet adhesion to adsorbed Alb was negligible when adsorbed Alb retained most of its native structure (<34% loss in alpha-helix), but began to linearly increase with the degree of adsorption-induced unfolding thereafter (r²=0.92). SDS-PAGE results showed that the platelet suspension was free of residual proteins and anti-Alb polyclonal antibodies completely inhibited platelet adhesion to adsorbed Alb, but had negligible effect on adsorbed Fg (used as a control); thus confirming that the platelets were adhering to adsorbed Alb and not some other residual protein in the system. Addition of an RGDS peptide to the platelet suspension strongly inhibited platelet adhesion to adsorbed Alb (~60% reduction on CH₃ SAM; p < 0.01), while the addition of RGES peptide had no inhibitory effect. Neutralization of arginine residues in the adsorbed Alb layer using 2,3-butanedione reduced platelet adhesion to a similar degree as exposing the platelets to the RGDS peptide in solution. These results indicate that the adhesion of nonactivated platelets to adsorbed Alb is primarily mediated by RGD-specific receptors and the degree to which the binding domains in adsorbed Alb are exposed and/or formed is directly proportional to the degree of adsorption-induced unfolding of the protein. Further studies will be carried out to identify the specific platelet receptors and Alb domains that mediate adhesion. These results indicate that we have much yet to learn about the mechanisms that influence platelet adhesion to adsorbed proteins.

8:00am Bi-TuM1 Molecular Simulation as a Surface Design Tool to Understand and Control Protein-Surface Interactions, R.A. Latour, Clemson University  INVITED
Surface-solute interactions are of great importance in a wide variety of applications in biomedical engineering and biotechnology, including medical implants, biocatalysis, immobilized-enzyme bioreactors, biosensors, bioseparations, and bioanalytical systems. While this is well recognized, very little is understood regarding how to design surfaces to optimally control protein adsorption behavior. To address this limitation, we are working on the development of molecular simulation methods to accurately predict protein-surface interactions at the atomic level. We have found that this type of molecular system is sufficiently unique that molecular simulation methods cannot simply be borrowed from other applications; but rather, they must be critically evaluated and often modified to accurately represent adsorption behavior.

8:40am Bi-TuM3 Surface-Induced Changes in the Structure of Beta-Helical Peptides, K. Fears, J. Kulp III, D.Y. Petrovykh, T. Clark, US Naval Research Laboratory
The stable structure of beta-helical peptides in solution provides a well-defined starting point for discerning the changes in secondary structure of peptides induced by surface adsorption. Understanding the adsorption of proteins on surfaces is of critical importance in medical- and biotechnology. The determination of the secondary and higher-order structure of adsorbed proteins, however, is challenging due to their inherent complexity. Peptides with simple secondary structures provide a good model for investigating the interactions between surfaces and the structural subunits of proteins. Beta-helical, rather than alpha-helical, peptides were selected for this study because of their stability and tendency to maintain a monomeric, unaggregated structure. Peptides were custom designed to switch between two different stable conformations as a function of solvent composition, as confirmed by nuclear magnetic resonance (NMR) and circular dichroism (CD) spectroscopies in solution. These peptides were adsorbed onto silica substrates from aqueous and organic solvents to determine their conformation post-adsorption.

9:00am Bi-TuM4 Enantiospecific Adsorption of Serine Enantiomers on the Chiral Cu[531] Surface, T. Eralp, A. Shavorsky, University of Reading, UK, D. Batchelor, BESSY and Universität Würzburg, Germany, G. Held, University of Reading, UK
The production of enantiopure chiral bio-relevant molecules is of significant importance for the development of new pharmaceuticals and the improvement of existing ones. In this context chiral surface systems, e.g. chiral molecules adsorbed on chiral single crystal surfaces, are of considerable interest as they are model systems for potential enantioselective heterogeneous catalysts or enantioselective selection. In this study the adsorption properties of L- and D-Serine enantiomers on the intrinsically chiral Cu[531] surface were investigated. These aminocids have four functional groups which can make bonds to the Cu[531] surface: OH, NH₂, and two oxygen atoms in the carboxyl group (-COOH). The geometry of the adsorption complex was characterised using XPS and NEXAFS. The bonding characteristics of the molecule strongly depend on the coverage. The main peak in the O1s XPS spectra, at BE 531.5 eV, is assigned to be overlapping signal of the two oxygen atoms in the deprotonated carboxylate (COO⁻) group forming bonds with Cu atoms. For the low coverages this peak has a shoulder at a lower BE (530.7 eV), as the coverage increases this shoulder disappears and a new peak appears at higher BE (532.8 eV). The low BE shoulder at low coverage is assigned to the oxygen atom also forming bond with the Cu atom. With increasing coverage the surface becomes more crowded and a less space-consuming configuration is assumed with a 'dangling' OH group, which is the origin of the high BE O1s peak.

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

The adsorption of protein to solid surfaces is typically accompanied by structural rearrangements as well as loss of bioactivity. These changes can be monitored by time-of-flight secondary ion mass spectrometry (ToF-SIMS) and the protein activity monitored by surface plasmon resonance (SPR). However, the ultra-high vacuum of the ToF-SIMS can alter the protein conformation. In this study ToF-SIMS was coupled with a variable temperature sample stage to monitor the conformational changes that occur when a surface-bound protein goes from a hydrated to a dehydrated state.

Changes in bioactivity of the surface bound proteins were investigated using SPR. Initial ToF-SIMS and SPR experiments were conducted on a surface-bound protein system of histagged humanized anti-lysozyme variable fragment (HuLys Fv) coordinated on a Ni²⁺-loaded nitrilotriacetic acid (NTA) surface. Continuing studies investigate fibrinogen (and fibrinogen fragments). Positive ToF-SIMS data from the protein surfaces were acquired with an ION-TOF TOF.SIMS 5-100 system (ION-TOF GmbH, Münster, Germany). Applying principal component analysis (PCA) to the ToF-SIMS data, the spectral differences resulting from two surface coverages and various heat treatments were determined. The spectra are separated into three groups: high protein coverage samples, low coverage below -80°C, and low coverage at -60°C and above. Trends observed in the plot suggest both surface coverage and heat treatment affected the secondary ion spectra. At the temperature below about -80°C, the protein molecules are frozen into their hydrated conformation. As the temperature is raised changes expose hydrophobic amino acid residues. The antigen binding capacity of surface-bound HuLys Fv before and after dehydration was measured by SPR. At the low coverage, the antigen binding capacity on the dried protein film was roughly 50% lower than that on the fresh film. As comparison, high coverage dried samples lost ~20% binding capacity. The loss of HuLys Fv bioactivity on the dried protein film was attributed to an irreversible disruption of protein native conformation during the drying process. The high coverage samples exhibited less loss of bioactivity, consistent with the smaller conformational changes observed by PCA. The use of the sugar trehalose as a protein stabilizer is also investigated.
NMR data acquired on polystyrene bead systems are in line with the determined peptide chain play a dominant role for the binding of the peptide. Solid state sequence are more bent away, indicating the leucines in the center of the calculated solution structure. Leucines in the center of the peptide are more probe individual leucine side chains of LK14 adsorbed onto a hydrophobic isopropyl group of each of the leucines, totaling 8 samples, was used to side of the helix and the hydrophilic on the other. Deuteration of the (K) and hydrophobic leucine (L) residues with an aerosol liquid chromatography (HPLC) method. We found that the VDPRPs were mostly contributed by the reservoir processes. We also found that characteristic features of the VDPRPs were determined by the monomer heating temperature and proposed possible mechanisms for the feature evolution. To evaluate the major substrate surface processes, we developed a quantitative analysis method using FTIR on both as-deposited PBLGs and chemisorbed PBLGs. Consequently, we were able to propose possible SI-VDP mechanisms leading to the surface-grafted PBLGs that were expected to have either high packing density with mostly α-helix segments or low packing density with a significant amount of both random coil and α-helix segments.

In this work, we have developed 11-mercaptoundecanoic acid (MUA)-polypeptide “bilayer” systems by adsorbing poly(diethylene glycol-l-lysine)-poly(l-lysine) (PEGIL-PLL) diblock copolyptide molecules of various architectures onto the bilayer side chains of the MUA SAM. Subsequently, we have developed a quantitative analysis method using FTIR on both as-deposited PBLGs and chemisorbed PBLGs. Consequently, we were able to propose possible SI-VDP mechanisms leading to the surface-grafted PBLGs that were expected to have either high packing density with mostly α-helix segments or low packing density with a significant amount of both random coil and α-helix segments.

In our study, we have developed an SI-VDP system having pressure and temperature control to reduce vacuum restriction 1000 times with high grafting efficiency and, thus, were able to synthesize grafted poly(gamma-benzyl L-glutamate)(PBLG) film of 167 nm thick under 0.75 mbar. More importantly, we quantitatively investigated mechanistic details of the SI-VDP process including monomer vaporization and reservoir polymerization in the monomer-receiver and monomer condensation and physiosorption and chemisorption polymerization on the substrate surface. To study the major monomer reservoir processes, we monitored the amount of vaporized monomers and developed a VDP reaction profile (VDPRP) method. In this work, we have developed 11-mercaptoundecanoic acid (MUA)-polypeptide “bilayer” systems by adsorbing poly(diethylene glycol-l-lysine)-poly(l-lysine) (PEGIL-PLL) diblock copolyptide molecules of various architectures onto the bilayer side chains of the MUA SAM. Subsequently, we have developed a quantitative analysis method using FTIR on both as-deposited PBLGs and chemisorbed PBLGs. Consequently, we were able to propose possible SI-VDP mechanisms leading to the surface-grafted PBLGs that were expected to have either high packing density with mostly α-helix segments or low packing density with a significant amount of both random coil and α-helix segments.

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Biomaterial Interfaces

Room: K - Session BI-TuA

Biofouling

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

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

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


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

4:20pm BI-TuA3 Emerging Strategies to Prevent Bacterial Colonization of Medical Biomaterials. J.D. Bryers, University of Washington, Seattle

Nosocomial (hospital-acquired) infections are the fourth leading cause of death in the U.S. with >2 million cases annually (or ~10% of American hospital patients). About 60-70% of all such infections are associated with an implanted medical device causing >$4.5 billion medical costs in 2002 and ~99,000 deaths annually. Over 65% of hospital-acquired infections are associated with implants or indwelling medical devices, with the case-to-fatal ratio being between 2-3:1. It is estimated that over 5 million artificial or prosthetic devices are implanted per annum in the U.S. alone. Microbial infections have been observed on most biomedical devices, including: prosthetic heart valves, orthopedic implants, intravascular catheters, artificial hearts, left ventricular assist devices, cardiac pacemakers, vascular prostheses, cerebrospinal fluid shunts, urinary catheters, ocular prostheses and contact lenses, and intrauterine contraceptive devices. Traditioal strategies to control medical device-based biofilm infections are based on the use of compounds that kill or inhibit the growth of freely suspended bacteria. However, "biofilm-bound" bacteria tend to be significantly less responsive to antibiotics and antimicrobial stressors than planktonic organisms of the same species. In fact, studies have shown that sub-lethal doses of antibiotics can exacerbate biofilm formation. Consequently, systemic antibiotic treatment typically fails to clear a biofilm infection and inevitably requires removal of the device. Moreover, the risk of antibiotic resistance development is drastically increased under the current standard use of systemic antibiotic treatment of medical-device infections. Here novel non-antibiotic based concepts in biomaterials design (novel stealth surfaces or biomaterials that biologically prevent bacterial colonization) will be presented.

4:40pm BI-TuA8 In situ Characterization of Barnacle Primary Cement Interfaces by ATR-FTIR Spectroscopy. D.E. Barlow, U.S. Naval Research Laboratory.

Barnacles adhere to surfaces by a proteinaceous cement, for which most studies to date have been ex situ analyses of the protein composition. However, very little is currently known about the chemical structure and composition in the original, undisturbed cement interfaces of barnacles (primary cement interfaces) that provide the strong adhesion to substrates in marine environments. We will present a method that has been implemented for characterizing primary cement interfaces of barnacles using in situ attenuated total reflection - Fourier transform infrared spectroscopy (ATR-FTIR). Primary cement of the barnacle Balanus amphitrite (= Amphibalanus amphitrite) was characterized without any disruption to the original cement interface, for settling and growing barnacles directly on double side polished germanium wafers. High quality IR spectra were acquired of live barnacle cement interfaces, providing a spectroscopic fingerprint of cured primary cement in vivo with the barnacle adhered to the substrate. Additional spectra were also acquired of intact cement interfaces for which the upper portion of the barnacle had been removed leaving only the base plate and cement layer attached to the substrate. This allowed further characterization of primary cement interfaces that were dried or placed in DI O. The resulting spectra were consistent with a proteinaceous cement, and allowed analysis of the protein secondary structure and water content in the cement layer. The estimated secondary structure composition was primarily β-sheet, with additional α-helices, turn, and unordered
components. The cement of live barnacles, freshly removed from seawater, was estimated to have a water content of 20% - 50% by weight. These results provide new insights into the chemical properties of the undisturbed barnacle adhesive interface. The ATR-FTIR method presented is also expected to be useful for *in situ* and *in vivo* studies of bioadhesives from other organisms.
Biomaterial Interfaces
Room: Hall 3 - Session BI-TuP

Biomaterial Interfaces Poster Session I

BI-TuP1 Formation of Stable Microbubbles by Encapsulation in Silica, K. Stoggs, University of New Mexico, G. Gupta, Los Alamos National Laboratory, M. Taris, New Mexico Tech, G.P. Lopez, University of New Mexico

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

BI-TuP2 Synthesis of Grafted PNIPAAm Surfaces Using ATRP in Presence of Air for Cell Adhesion Studies, P. Shivapooya, L.K. Ista, S. Mendez, G.P. Lopez, University of New Mexico

Poly(N-isopropylacrylamide) (PNIPAAm) is a thermoresponsive polymer that exhibits a change in relative hydrophobicity above and below its lower critical solution temperature (LCST ~ 32°C). This stimul responsive polymer when grafted onto surface can act as a biofouling resistant coating as it has the property of formation and collapse of hydrogen bonding between water molecules below and above the LCST. Atom transfer radical polymerization (ATRP) has been proven to yield well-defined polymer but in rigorously deoxygenated environment, for example in a Schlenk line or glove box. Matijaszewski et al. group developed activators regenerated by electron transfer (ARGET) which allow ATRP in presence of limited amount of air (Langmuir 2007, 23, 4526-4531). We report grafted PNIPAAm brushes synthesized by this relatively simple method of ARGET ATRP which does not require an oxygen-free environment using a small amount of reducing agent together with a catalyst under homogeneous conditions. We evaluated the effect of the amount of reducing agent and reusability of monomer solution on the grafting thickness. The grafted PNIPAAm surfaces have been characterized by FTIR, XPS, contact angle measurements and ellipsometry. We anticipate using these grafted PNIPAAm surfaces for cell adhesion studies above and below the LCST.

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

The extracellular matrix, or ECM, remains a hidden biological interface between a cell and a substrate. One method for examining proteins, such as enzymatic digestion and physical scraping, damage the ECM and dissociate the cells from the surface. Traditional cell harvesting methods, including syringe pumps and sealing devices are required, because most microbubbles must be formed under these types of gases. This requirement limits the conditions under which microbubbles can be studied. They cannot be studied on a bench top open to atmosphere. Once microbubbles have undergone soft petrification, they can be studied in a wide range of environments. In soft petrification of air filled lipid coated microbubbles, a vapor deposition technique encapsulates the microbubbles in a thin silica shell. After undergoing soft petrification, it was observed that microbubble stability under adverse conditions is significantly improved. These adverse conditions include pressures up to 120 psig and temperatures up to 80°C. Those values are double the values found for microbubbles without the silica coating. Other unique characteristics of the microbubbles include their ability to withstand temperatures well below freezing, without loss of size. Air filled microbubbles have a bench top life span of approximately four hours before encapsulation. Following encapsulation, it is observed that the same microbubbles have a bench top lifespan of up to several months. Several formulations were characterized and unprecedented air filled microbubble studies are facilitated by this technique.

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

Protein immobilization on surfaces is important in many areas of research, including their biophysical characterization, affinity chromatography, and heterogeneous immunosassays. We describe a new site-specific C-terminal immobilization of proteins on surfaces to control the orientation and attachment point of the protein. Our strategy was to exploit in vitro chemistry and the related hydrazine attack reaction. To provide-proof-of principle of this approach, a tripartite thioedoxin-intein-elastin-like polypeptide (ELP) fusion was synthesized in E. coli from a plasmid-borne gene. In this fusion protein, the C-terminus of the target protein thioedoxin was fused with the N-terminus of an intein, a self-splicing protein domain. An ELP was fused to the C-terminus of the intein to facilitate purification of the fusion protein because ELP fusions allow simple purification of proteins from cell lysate by in vitro, salt-triggered phase transition of the ELP. Immobilization of the intein at the C-terminus of the target protein provided an unnatural peptide bond between the target protein and the intein, which is selectively reactive towards nucloephiles such as hydrazine. In order to immobilize the target protein – thioedoxin– a hydrazine-functionalized surface on a protein-resistant background was fabricated by modifying a gold-coated glass substrate with a mixed self-assembled monolayer consisting of a hydrazine-terminated hexa(ethylene glycol) thiol and a tri(ethylene glycol)thiol. We demonstrate that the hydrazine groups on the surface were able to directly attack the unnatural peptide bond between the target protein and the intein, liberating the thioedoxin from the tripartite fusion and covalently attaching it to the surface. As a result, the target protein was site-specifically immobilized on flat minor substrates with no function. This technique provides many advantages over other site-specific immobilization methods, including faster reaction kinetics, higher surface density, and the ability to easily purify the protein prior to immobilization though a simple, one-step non-chromatographic process that exploits the phase transition of the ELP.


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

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

Low column efficiency is a common problem associated with the affinity purification of surfactant solubilized membrane proteins synthesized in recombinant and cell free expression systems. Elastin-like polypeptide (ELP) tags, which have been designed to allow non-chromatographic purification of soluble proteins, offer a potential means to enable facile large-scale purification of detergent solubilized recombinant membrane proteins. However, the phase transition temperature (Tc) of ELPs is sensitive
to the addition of cosolutes and many detergents increase the $\zeta$ of ELPs to temperatures greater than the thermal denaturation temperature of many proteins, which are not stable to the ELP, hence promoting their purification. To identify detergents that would satisfy the dual and potentially conflicting requirements of stabilizing membrane proteins fused to an ELP, we screened different detergents with respect to their effect on the $\zeta$ of ELPs (Va, 1.9-1.8). We found that dodecyl maltoside (DDM), a detergent that is commonly used to solubilize reconstituted membranes, did not significantly alter the phase transition characteristics of ELPs or their structure as probed by a temperature-programmed turbidity assay and circular dichroism spectroscopy. Our results clearly indicate that DDM does not affect the inverse transition cycling of ELPs and therefore may be useful to purify membrane proteins which are otherwise difficult to extract and purify by affinity chromatography.

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

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


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

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

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

Scaffolds [5% poly(ethylene) (PCL)] were prepared using thermally induced phase separation. Samples were plasma polymerized in a custom-built radio frequency reactor using heptalamine (H7A) for 60s and 60s. Additional scaffolds were prepared by spincoating PCL on Si wafers. For each of the four treatment conditions, surface homogeneity was confirmed across the wafers and scaffold sections via XPS. Surface topography was investigated on wafers by AFM, and scaffolds were examined using SEM.

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

Polymer aggregates formed on Si following PPHA treatment at 20W, but were diminished at 50W. Topographical changes were more pronounced on PPHA PCL-coated Si. Although spherulite size variation was minimal between untreated semi-crystalline PCL and PPHA PCL on Si for most treatments, complete recrystallization of the base polymer was observed at 50W. 60s. Change in surface roughness was evidenced by disappearance of well-defined fibrous domains as treatment power and time increase for all samples, except at 50W 60s when distinct spherulites re-emerged. Highly reactive HA recombination led to aggregate size reduction on Si, whereas PPHA on PCL appeared to affect structural organization of the substrate. Scaffold morphology also changed following PPHA, as more energetically favored fibrous extensions in the porous region of the surface were observed by SEM. These results may be used in conjunction with cellular studies to tune PPHA reactions on scaffolds as required for support of various cell types.

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

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

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

The results from these studies are enabling us to assess the suitability of currently available molecular simulation methods and force fields and are providing us with a better understanding of peptide-surface interactions at the atomic level.
BI-TuP13  ToF-SIMS Study of Fibronectin Orientation on Self-Assembled Monolayers, L. Árnadóttir, J. Brison, L.J. Gamble, University of Washington

Protein adsorption and orientation plays a critical role in many biomedical applications. Fibronectin (FN) is an extra cellular matrix protein that is involved in many cell processes such as adhesion, migration and growth. The orientation and conformation of FN adsorbed onto surfaces can therefore have a critical effect on cell-surface interactions. In this study the adsorbed orientation and conformation of the 7-10 fragment of FN (FNIII\textsubscript{7-10}) was studied on four different model surfaces (self-assembled monolayers (SAM) of C\textsubscript{11} alkanethiols on Au, -CH\textsubscript{3}, -NH\textsubscript{2}, -COOH and -OH terminated SAM). The FNIII\textsubscript{7-10} fragment incorporates both the Arg-Gly-Asp (RGD) FN receptor binding motif and the PHSRN synergy site which participate in the RGD binding. The effect of different surface chemistries on binding and adsorption configuration was investigated using X-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectrometry (ToF-SIMS). XPS showed significantly higher protein coverage on the hydrophobic -CH\textsubscript{3} terminated surface than the hydrophilic and negatively charged –COOH terminated surface. Both XPS data and SIMS peak intensities for certain amino acid were used to verify the adsorption process and monolayer coverage. Full coverage was determined by a plateau in SIMS peak intensities with increasing protein exposure. A principal component analysis (PCA) of the ToF-SIMS data on surfaces with similar sub-monolayer protein coverage was then used to determine changes in the spectra that differed when the fragment was adsorbed on various surfaces. These results are related to different orientation or conformations of the fragment on the different surfaces.

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

Calcium phosphate-based ceramics are used in medicine and dentistry due to excellent biocompatibility with human tissues. The formation of hydroxyapatite coatings with a porosity gradient on the surface of metal implants can provide a surface suitable for the in growth of connective tissue. A need exists to both protect the metal substrate against corrosion and provide sufficient mechanical adhesion. Hydroxyapatite coatings have been produced by the reactive, radio-frequency sputtering of stoichiometric Ca\textsubscript{10}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2} targets using planar magnetrons onto titanium-coated silicon substrates. A fully dense and thin initial-coating layer appears to avoid cracking, improves substrate adhesion, and provides corrosion protection (J. Mater. Res. 16, 2001, 3238). The effect of varying the wear rate is now evaluated on the mechanical behavior of sputter deposited hydroxyapatite coatings.

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

We demonstrate the impact that intra-molecular and nucleotide-gold interactions have on conformation of surface-immobilized DNA probes and their hybridization behavior. We take advantage of a method based on the intrinsic affinity of adenine nucleotides for gold (Opdahl et al., PNAS, 104, 9–14, 2007) to immobilize block-oligonucleotides having sequences that follow a d(A\textsubscript{k}-T\textsubscript{m}-N\textsubscript{n}) pattern: a block of \(k\) adenines [d(A\textsubscript{k})], followed by a block of \(m\) thymines [d(T\textsubscript{m})], and a block of \(n\) (arbitrary) nucleotides [d(N\textsubscript{n})]. Such block-oligos assemble on gold via the d(A) blocks, which allow a high degree of control over DNA surface coverage and conformation. We characterize immobilization and hybridization of these DNA probes using x-ray photoelectron spectroscopy (XPS) and surface plasmon resonance (SPR) imaging, specifically to compare two 15-nucleotide N\textsubscript{5} sequences: 15 thymines (T\textsubscript{15}) and a realistic arbitrary sequence (P\textsubscript{15}). For A15-T5-P15 probes, the hybridization efficiency at the P15 end is enhanced 2-fold compared to that at the A15 end. For A15-T20, which forms a hairpin in solution, we observe a larger asymmetry of hybridization efficiencies when immobilized on gold: while the T15 end is highly accessible for hybridization, the A15 end has virtually no hybridization activity. A thiolated version of the same probe (A15-T20-SH), in contrast, exhibits little asymmetry and overall low hybridization efficiency with either A15 or T15, consistent with an immobilized structure of a stable hairpin. Additional experiments, whereby a displacement thiol is added to reduce DNA-gold interactions, are used to support our inferences about the role played by intra-molecular and surface interactions in immobilization and hybridization of DNA probes.
Wednesday Morning, November 11, 2009

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

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

8:00am BI+AS+BM+MS-WeM1 Design of Antibody Array-Based Sensors for Disease Proteomes: Grand Challenges, C. Wingeren, Lund University; Sam Dubai, DNATED

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

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

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

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

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

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

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

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

This talk will highlight recent work from my laboratory that adapts new interfacial technologies to suppress noise (N) and amplify signal (S) leading to heterogeneous assays with extraordinarily high S/N. In the first demonstration, I will focus on the adventitious adsorption of proteins as the primary factor that controls the limit-of-detection (LOD) of protein microarrays and limits the measurement of analytes from complex mixtures such as serum or blood. I will show data on a new protein microarray assay where background adsorption is effectively eliminated through the use of a protein-resistant –nonfouling– polymer brush. These “zero background” protein microarrays were successfully used to quantify protein analytes in serum with femtomolar LOD and a dynamic range of 6 orders of magnitude of analyte concentration. These LODs are 100-fold lower when compared to the same protein microarrays spotted on a conventional polymer substrate that displays high binding capacity but significant adventitious protein adsorption. This study also provided the first demonstration of the interrogation of an analyte directly from undiluted, whole blood by a protein microarray with a LOD of ~15 fM. Next, I will summarize recent work in my laboratory on the development of a new isothermal fluorescence signal amplification and detection scheme that exploits the high-density of terminal thiol groups in TdT-added up to 100 fluorescent nucleotides to the end of a short DNA tag with an exposed 3’-OH. I will show how DNA microarrays that are printed on the nonfouling polymer brush exhibit low background signal, yet allow on-chip fluorescence signal amplification, leading to DNA microarrays that exhibit a sub-picomolar LOD, which appears to be the lowest LOD reported for DNA microarrays, to date.

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

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

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

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

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

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

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

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

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

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

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

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

In addition to probing specific binding of ligands to membrane receptors, we showed that nanoplasmonic sensors provide a unique means to probe biomolecular structural changes, such as during the formation of a SLB from adsorption and rupture of lipid vesicles.[1]

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

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

References:

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

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

We will present how transfer of biomolecules between an SLB and liposomes can be monitored in real-time giving more insight into the complex mechanism of transfer including influence of electrostatic interaction, ionic strength, phase and molecular structure of lipids, as well as time scale of the transfer process. Recent results show that the interaction process consists of an attachment-transfer-detachment (ATD) sequence, where added liposomes first attach to a preformed SLB, then transfer lipid molecules and eventually detach, leaving behind a compositionally modified SLB and ditto vesicles.[1] We will demonstrate how the ATD process can be used for changing modifications, e.g., changing the membrane composition, e.g., for the formation of a highly stabilized (SDS-resistant) lipid monolayer on TiO₂, which can then be used for the reassembly of an SLB.[2] We propose this as a promising method for in situ preparation of asymmetric SLBs.

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


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

This talk demonstrates that pores with diameters below 50 nanometers make it possible to detect enzyme reactions, molecular phases transitions, and nanoscale self-assemblies in situ and in real time. For instance, coating the inner walls of nanopores with self-assembled lipid bilayers, afforded controlled shrinkage of this pore to a size that made it possible to detect individual proteins. Remarkably, the extent of pore shrinkage could be...
controlled with sub-nanometer precision by the chain lengths of the acyl chains on the lipids that were chosen to assemble the bilayer. Due to the extreme sensitivity of single-channel recording of ion currents through nanopores, this approach made it possible to monitor molecular changes and rearrangements of the lipid bilayer. These changes included phase transitions, variations in membrane composition, and enzymatic reactions on membranes. For example, this approach made it possible to monitor the activity of atotmol amounts of phospholipase D (PLD) and phospholipase C (PLC) – two membrane-active enzymes that are critical for cell signaling.

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

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

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

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


The interaction between cell and extracellular matrix (ECM) governs multiple cellular functions and contributes to promote inflammation and tumor metastasis. Therefore, cellular behavior needs to be monitored in the ECM interactive circumstance. Most of previous studies on cell adhesion are based on immunofluorescence microscopy. For cell adhesion dynamics studies, label-free optical techniques that can monitor continuously cell-ECM interfaces for living cells are required. Here we developed surface plasmon resonance imaging ellipsometry (SPRIE) which can simply image cell-ECM interfaces for live cells with high contrast and at real-time. To visualize cell adhesions to ECM, null-type imaging ellipsometry technique with the attenuated total reflection coupler was applied and both of transverse magnetic and electric waves were made use of. These characteristics make it possible to acquire the high contrast image of cell adhesions. Different features and dynamics of cell adhesion patterns in ~ 100 nm cell-ECM interfaces were observed for A10, human coronary artery smooth muscle cell hCASMC, and human umbilical vein endothelial cells (HUVEC) on fibronectin and collagen ECM layers with 1 μm spatial resolution and 30 sec time interval upto 3 days. Harmonized changes of entire adhesion proteins were observed during cell division and cell migration through our imaging system without any labeling. SPRIE images were compared with confocal fluorescence microscopic images of cell adhesion proteins for validation of SPRIE images. Preliminary results on SPRIE studies on the effect of shear force on cell adhesion and migration will be also discussed.

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

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


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

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

Red blood cell (RBC) membrane fluctuation mediated by cooperative relationship between its cytoskeleton and lipid bilayer plays an important role in protein dynamics that is indicative of structural-functional properties of healthy or diseased RBCs. Probing of this characteristic membrane behavior requires dynamic interrogation of RBCs under physiological conditions by high-resolution, noninvasive microscopy techniques for which RBCs are required to be immobilized on a substrate while maintaining their viability. Therefore, detailed understanding of the adhesion process and its consequence on RBC shape and dynamic membrane response is critical. In the present study, we demonstrate our ability to engineer substrates with tunable surface zeta potential (SZP) for precise control of RBC adhesion. Specifically, 10 nm gold nanoparticles are adsorbed on poly-L-lysine coated cover slip as a compliant layer to locally modify the non-specific interaction between RBC membrane and substrate.

By combining scanning probe microscopy (SPM) and differential interference contrast (DIC) imaging techniques we develop a quantitative measurement methodology to investigate the relationship between attachment strength, RBC morphology, cell membrane fluctuation on these charge and topographically modulated substrates. Adhesion-induced tending of the RBC membrane on modified substrates leads to changes in cell shape and functionality as determined by SPM force-volume and DIC monitoring of membrane dynamics. The substrate preparation and measurement methods presented here provide a feasible platform to obtain structure-function relationships of viable RBCs under physiological conditions and with that allow us to investigate dynamic behavior of RBCs and their response to diseases.
medical diagnostic instruments; extreme ultra-violet (EUV) radiation generation; and explosive detector calibration. This paper illustrates some of the manufacturing and instrument applications of ink-jet technology.

8:40am 

9:20am 

10:40am 

11:00am 

References


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

"In this slide format2,3. Here, we have formed these polymer microarrays for the first time using ink-jet printing, to offer flexibility of slide production. Characterization was achieved using a high throughput surface analysis approach, including the techniques of X-ray photoelectron spectroscopy, time-of-flight secondary ion mass spectroscopy and sessile drop water contact angle measurements2. Of particular interest were polymers containing ethylene glycol functionality that were investigated for their switchable properties under biologically relevant conditions."

"A method for forming polymer microarrays has been developed whereupon a contact printer is used to deposit nanolitre volumes of premixed acrylate monomer and initiator to defined locations of a glass substrate. Polymeric, or plastic, biochips have several advantages in cost, problems by utilizing the steadily maturing art of inkjet printing on polymer substrates. Polymeric, or plastic, biochips at a cost-effective industrial scale."

"The platform is used to ensure print quality and an on-board vision system enables the visualization of the homogeneous distribution of the drug. These results are a promising first step to ink jet printing of pharmaceuticals."

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"In this presentation we will review the use of inkjet printing to fabricate Micro-Electro-Mechanical Systems (MEMS). We are investigating the use of sintered silver nanoparticle inks for the structural layer and polymers for the sacrificial layer in printed MEMS fabrication. As an example, inkjet printing technology has been used to fabricate microwave transmission lines for a glass substrate (with MEMTronics Corporation). 50 mm resolution was obtained using 10 pl drop volumes on a Corning 7740 glass substrate. The conductivity of the sintered silver structures were 1/6 that of bulk silver after sintering at a temperature much lower than the melting point of bulk silver. A comparison of the DC resistance of the sintered silver shows that it can match the performance of electroplated and sintered copper. Printed copper lines demonstrated a return loss of 1.62 dB/cm at 10 GHz and 2.65 dB/cm at 20 GHz. We will also discuss printing MEMS hot-wire anemometer sensors for use in aeronautical applications (with Tao of Systems Integration)."
9:40am 11+B1+MN+SE+AS+ThM12  Study on the Effects of Particle Size and Substrate Surface Properties on the Deposition Dynamics of Inkjet-Printed Colloidal Drops for Printable Photovoltaics Fabrication, S. Biswas, Y. Sun, Binghamton University

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

Thin Film Room: B4 - Session TF-ThM

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

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

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

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

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

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

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

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


TiO2 and Fe2O3 nanoparticles are of significant importance in both chemical and biological applications. TiO2 nanoparticles are used in paint, coatings, food, solar technology and many other areas. Fe2O3, as well as other magnetic nanoparticles, are used in the biomedical industry in drug delivery schemes as well as for magnetic resonance imaging contrast agents. Silica-coated TiO2 and Fe2O3 nanoparticles have distinct properties and enhanced functionality over those of uncoated nanoparticles. Plasma-enhanced chemical vapor deposition (PECVD) was employed to conformally coat TiO2 and Fe2O3 nanoparticles with SiO2 and amine-containing films, thereby creating composite nanomaterials. Hexamethyldisiloxane (HMDSO)/O2 plasmas were used to create SiO2 and SiO2C2H2-coated nanoparticles and pulsed hexylamine (HexAm) plasmas were used to create amine-containing hydrocarbon materials, all of which were analyzed using Fourier-transform infrared spectrosocopy (FTIR), x-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), and magnetic susceptibility. Films deposited on the nanomaterials showed little difference from those deposited on flat substrates. The performance of the TiO2 nanoparticles was tested using UV-vis spectroscopy to determine dispersion characteristics of SiO2-coated TiO2 materials, which can give information about the agglomeration of the nanoparticles in solution. Notably, the coated materials stay dispersed longer in polar solvents, suggesting the coated nanoparticles may be better suited for applications involving colloidal suspensions. Magnetic susceptibility characterized the magnetic properties of the Fe2O3 nanoparticles before and after film deposition. Comparison of the mechanical and chemical properties of different composite nanomaterials will be discussed along with the influence of film composition on performance.
10:40am TF-ThM9 Synergistic Ag (111) and Cu (111) Texture Evolution in Phase Segregated Cu_{1-x}Ag_{x} Magnetron Sputtered Composite Thin Films. D.I. Filoti, A.R. Bedell, J.M.E. Harper, University of New Hampshire

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


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

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

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

Moderator: G.J. Leggett, University of Sheffield

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

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

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

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

Polyethylene glycol) (PEG) can be incorporated into the membrane of liposomes through lipid molecules end-functionalyzed with a PEG chain and these liposomes were shown to spontaneously fuse to PEG-SLB on glass surfaces with a highly hydrated PEG cushion on each side of the membrane allowing ample space and protection for incorporation of membrane proteins. Since Cremer and coworkers also showed that with increasing PEG concentration the air-stability of PEG-SLBs increases it is a very promising SLB system to use for spotting where membrane air exposure during processing is a severe constraint. We present an investigation of the limits to spontaneous PEG-SLB in terms of PEG-lipid density, demonstrating that crossing the mushroom-to-brush regime of polymer concentration prevents the PEG-SLB formation due to steric effects and shielding of the interactions. Furthermore, we present conditions under which formation of PEG-SLBs is facilitated and can proceed by liposome fusion also in the brush regime as well as characterization of the kinetics of formation and the structure of these PEG-SLBs. The use of such buffers and liposomes for production of membrane arrays on glass using a non-contact piezo-spotter was then explored in detail in order to find optimal conditions of buffer composition and PEG concentration.


3:00pm BI+AS+NS-ThA4 Direct Laser Patterning of Soft Matter: Photothermal Processing of Supported Phospholipid Multilayers with Nanoscale Precision. M. Mathieu, S. Kaufmann, S. Syed, S. Buergel, J. Voeroes, ETH and University Zurich, Switzerland

Photothermal patterning constitutes a powerful tool for micro- and nanopatterning of phospholipid films. Recently, these coatings have also proceeded by liposome fusion also in the brush regime as well as conditions under which formation of PEG-SLBs is facilitated and can occur by liposome fusion also in the brush regime as well as characterization of the kinetics of formation and the structure of these PEG-SLBs. The use of such buffers and liposomes for production of membrane arrays on glass using a non-contact piezo-spotter was then explored in detail in order to find optimal conditions of buffer composition and PEG concentration.

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

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

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

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

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

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

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

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


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

Advances in Microfluidics for BioMEMS Moderator: G.W. Rubloff, University of Maryland
2:00pm BM+MN+MS+TF+BI-ThA1 Advances towards Programmable Matter, D. Erickson, Cornell University INVITED

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

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

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

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

The development of pH sensitive, biocompatible block copolymer vesicles has enabled the intracellular delivery of water soluble drugs and proteins. Improving the encapsulation efficiency of the vesicles is now a critical parameter. Transferring the production method to a microfluidic device creates the potential to vary the encapsulation conditions and improve this efficiency. In this work, a flow focussing microfluidic device is used. The self assembly of PMPC-b-PDPA block copolymer vesicles is induced within the device by changing the pH of the vesicles within the microchannels. The use of pH shift eliminates the need for organic solvents currently required for glass capillary production methods. This enables the biocompatibility of the block copolymers to be maintained, an essential factor for their application as molecular delivery vehicles. The flow focussing microfluidic device was produced through standard soft lithography techniques. A three-channel flow system is used with the copolymer in solution at pH6 in the central channel and aqueous buffered solutions flowing in the channels either side. The laminar flow conditions within the microfluidic device result in a pH gradient at the interfaces where the three channels meet and where the block copolymers self-assemble into vesicles. These vesicle formation processes have been imaged using confocal microscopy with FRET with a block copolymer containing both rhodamine and fluorescein isothiocyanate groups. Dynamic light scattering and TPEF were used to confirm vesicle formation. With 50nm to 250nm vesicles continuously being produced within the device it was then possible to investigate whether higher encapsulation efficiencies can be achieved using the microfluidic device. The protein myoglobin was introduced through the central channel along with the copolymer. Spectrophotometric analysis indicated the overall the efficiency of the encapsulation process within the device is not a significant improvement on the standard bulk methods currently used, involving sonication of the vesicle solution containing the molecule to be encapsulated. Despite this, the continuous nature of microfluidic devices, as well as the lack of organic solvents being used in the production process indicates that the development of these devices offers a viable alternative production method for polymer vesicles that may enable the increases in encapsulation efficiency to be achieved. Work is ongoing to achieve this using the same pH shift mechanism within a glass capillary microfluidic device.

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

Analysis of biomolecule interactions based on surface plasmon resonance (SPR) microscopy provides a label-free approach to monitoring arrays of biomolecule interactions in real time. Typically the microarray sensing surfaces have been prepared ex situ. Here, we demonstrate the capability of a multi-channel microfluidic flow cell array (MFCA) to expand the throughput capability when integrated with SPR microscopy. In addition, the MFCA provides an in situ approach to array fabrication that allows full characterization of the biomolecule immobilization process. We use the MFCA for delivery of sample solutions with continuous flow in 48 channels in parallel for rapid microarray creation and binding analysis while using SPR microscopy for real-time monitoring of these processes. Label-free measurement of antibody-antibody interactions demonstrates the capabilities of the integrated MFCA-SPR microscopy system and establishes the first steps of the development of a high-throughput, label-free immunogenicity assay. We demonstrate a limit of detection (LOD) of ~80 ng/ml for the particular antibody pair we studied. This LOD is ~6 times lower than the industry recommended immunogenicity assay detection limit. The high-throughput nature of the integrated system allows a large number of replicate experiments, including control experiments, to be performed simultaneously on the same sensor surface in a short time. The integrated system also will be applicable for more general high-throughput protein-array based analysis.


Nanochannels with a diameter of about 100nm are a novel method for stretching DNA for genomic investigations. Such devices are implemented through standard nanolithography in fused silica. The elongation of DNA results from an interplay of steric and entropic effects. Previous applications of nanochannel stretching included sizing, restriction mapping, and observation of transcription factor binding. We will discuss the basic operation of these technique, and the application to artificial substrates with predefined epigenetic marks.

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

A novel microfluidic device was designed in order to generate stable, quantifiable concentration gradients of biomolecules in a cell culture chamber of 2-D and 3-D shapes and sizes such as endothelial cells. Endothelial cells form the inner lining of blood vessels and initiate a critical step in angiogenesis (the sprouting of new blood vessels) during wound healing and cancerous tumor growth. Therefore, a deeper understanding of the critical biochemical and biochemical factors regulating endothelial cell sprouting can lead to improved clinical therapies for a wide range of diseases. Microfluidic devices provide rapid control of soluble growth factors inside the microfluidic cell culture chamber was determined by simulation and experiment, and the stability of the gradient was verified over multiple hours. This device allows independent tuning of the matrix rigidity, the growth factor absolute concentration, and the growth factor concentration gradient steepness within a single experimental platform.
Sprout formation of dermal microvascular endothelial cells was studied within collagen gels of varying density (0.3 - 2.7 mg/mL, corresponding to shear moduli of 8 – 800 Pa) that contained stable gradients of soluble vascular endothelial growth factor (VEGF). These experiments revealed that endothelial sprouting into multi-cellular, capillary-like structures is optimized at an intermediate collagen matrix density (G’~100 Pa). At lower matrix densities, cells were more likely to lose their coordinated motion and migrate as individual cells through the matrix; while at higher matrix densities, the cells formed broad cell clusters that rarely elongated into a sprout. Sprout thickness directly correlated with matrix rigidity, with thicker and less frequent sprouts present in gels with the highest shear moduli. Intriguingly, our 3D experiments also found that endothelial sprouts alter their sensitivity to VEGF depending on the matrix density, suggesting a complex interplay between biochemical and biomechanical factors. As matrix stiffness increases, steeper VEGF gradients and higher VEGF absolute concentrations are required to induce directional sprouting. In more compliant gels, endothelial sprouts that originally misaligned were able to turn and properly reorient parallel to the VEGF gradient; however, this turning phenomenon was only rarely observed in stiffer gels. These results demonstrate that matrix stiffness is an effective factor in stabilization and orientation of endothelial cells during sprouting and suggests new anti-angiogenic strategies for potential cancer treatment as well as pro-angiogenic strategies for regenerative medicine scaffolds.

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

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

BI-ThP2 Aqueous Polymer Nanografting: AFM patterning of Poly-L-lysine on Oxide Surfaces

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

BI-ThP3 High Sensitivity Electrochemical Immunosensor Based on Plasma Modified TiO2/C-Chitosan

We present a scanning probe lithography technique that allows for patterning of adsorbed, water-soluble polymers on functionalized oxide surfaces. SiO2 and borosilicate glass surfaces were functionalized with a negatively charged carboxyl-terminated silane monolayer. A 2 nm poly-L-lysine layer was then deposited over the silane film. An atomic force microscope (AFM) probe was used to write lines and areas of the positive poly-L-lysine layer, exposing the negatively charged silane underlayer. The AFM scribing experiments were performed both in air and in water. Regions were scribed and then backfilled with a fluorescently tagged polymer. Characterization of the scribing was done with tapping mode AFM. Optical fluorescence microscopy was used to image backfilled regions. AFM height and phase mode data showed lines and spaces with half pitch features as small as 12 nm created with a scribing force of 0.3 μN.

BI-ThP4 Measuring Magnetic Properties of Individual Magnetosomes by Scanning Transmission X-ray Microscopy

We have studied the Fe 2p X-ray magnetic circular dichroism (XMCD) of individual magnetosomes - biominalerized ferrimagnetic nano-crystals in magnetotactic bacteria (MTB) - using scanning transmission X-ray microscopy (STXM). Magnetosomes are intracellular magnetite (Fe3O4) or greigite (Fe3S4) nano-crystals (typically 30-60 nm in size), enclosed in a lipid membrane. A chain of magnetosomes is used by MTB to orient within a variety of environments. Our initial goal, which has been achieved, was to demonstrate that the STXM has the capability to investigate magnetic properties of sub-50 nm areas in biological systems. The Fe 2p XMCD of individual Fe3O4 magnetosomes of MV-1, a marine vibrio species of magnetotactic bacteria, was measured with the sample at 30 degrees relative to the beam to sense the in-plane magnetic component. This is the first such measurement of the XMCD of a single magnetosome to our knowledge. Evidence for multiple domains was found for some magnetosomes. In addition we have begun to explore the associated biochemistry by STXM spectromicroscopy at high spatial resolution (30 nm) at the C 1s and O 1s absorption edges. The combined XMCD and biochemical imaging will help further the understanding of biominalerization processes present in MTB and other environmental organisms.

Research funded by NSERC. The Canadian Light Source is supported by NSERC, NRC, CHIR, and the University of Saskatchewan. Some measurements were also made at STXM11.0.2 at the Advanced Light Source, which is supported by the Division of Basic Energy Sciences of the U.S. Department of Energy.

BI-ThP6 A Novel Technique for the Determination of Orientation in Atomic Force Microscopy

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

BI-ThP8 Characterizing the Carbohydrate Microarray: XPS, ToF-SIMS, SPR, and ELLA Analysis of Glycan-Modified Surfaces

We have studied the Fe 2p X-ray magnetic circular dichroism (XMCD) of individual magnetosomes - biominalerized ferrimagnetic nano-crystals in magnetotactic bacteria (MTB) - using scanning transmission X-ray microscopy (STXM). Magnetosomes are intracellular magnetite (Fe3O4) or greigite (Fe3S4) nano-crystals (typically 30-60 nm in size), enclosed in a lipid membrane. A chain of magnetosomes is used by MTB to orient within a variety of environments. Our initial goal, which has been achieved, was to demonstrate that the STXM has the capability to investigate magnetic properties of sub-50 nm areas in biological systems. The Fe 2p XMCD of individual Fe3O4 magnetosomes of MV-1, a marine vibrio species of magnetotactic bacteria, was measured with the sample at 30 degrees relative to the beam to sense the in-plane magnetic component. This is the first such measurement of the XMCD of a single magnetosome to our knowledge. Evidence for multiple domains was found for some magnetosomes. In addition we have begun to explore the associated biochemistry by STXM spectromicroscopy at high spatial resolution (30 nm) at the C 1s and O 1s absorption edges. The combined XMCD and biochemical imaging will help further the understanding of biominalerization processes present in MTB and other environmental organisms.

Research funded by NSERC. The Canadian Light Source is supported by NSERC, NRC, CHIR, and the University of Saskatchewan. Some measurements were also made at STXM11.0.2 at the Advanced Light Source, which is supported by the Division of Basic Energy Sciences of the U.S. Department of Energy.

BI-ThP9 Interferometric Lithography of Self-Assembled Monolayers

Self-assembled monolayers (SAMs), especially alkanethiols on gold, have been extensively used as model system for studying surface modification strategies. In this work, we utilize this platform to fabricate carbohydrate-modified biosensors composed of mixed monolayers of mannose headgroups and oligo(ethylene glycol) (OEG) moieties on gold. We have extensively used x-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), surface plasmon resonance (SPR), and enzyme-linked lectin assay (ELLA) to understand the composition, structure and reactivity of these mixed SAMs to carbohydrate-binding proteins (lectins). XPS and ToF-SIMS results give a clear indication that the composition of mannose on the surface is directly proportional to its molar ratio in solution. However, when these sensors are exposed to lectins, XPS shows that the amount of protein is inversely proportional to the amount of mannose present on the surface. We performed SPR studies to obtain a quantitative comparison of the amount and multivalent binding of lectins on these mixed SAMs. Detailed study of this system using XPS, ToF-SIMS, SPR and ELLA suggests that an optimum density of mannose on the surface is required to improve the sensitivity and stability of these sensors.
lithography (IL) is rapid, and uses minimal instrumentation. IL using a cw, 244 nm frequency doubled Ar-ion laser source has been found to yield structures as small as 5 nm using SAMs of macroscopic extended areas. SAMs of alkanethiols on gold may be photo-oxidized to yield weakly bound sulfonates that may be displaced by solution-phase adsorbrates to yield patterns of chemical composition. Here, we demonstrate the fabrication of patterns of surface free energy with a period of 200 nm. Protein adsorption may be controlled by using IL to selectively photodegrade oligo(ethylene oxide) (OEG) terminated SAMs of alkanethiols on gold and of trichlorosiloxanes on glass. Nanopatterned streptavidin formed this way retains its ability to bind biotinylated proteins. Finally, monolayers of phosphonic acids on titanium dioxide may be readily patterned and used as templates for the fabrication of a variety of architectures, including 35 nm TiO2 structures on glass. IL is an inexpensive and convenient method of producing molecular nanostructures over square centimetre and larger areas.

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

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


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

References:


We have developed a new technique for on-chip, isothermal signal amplification using terminal deoxynucleotidyl transferase (TdT), a template-independent DNA polymerase that catalyzes the sequential addition of deoxynucleotides (dNTPs) at the 3'-OH group of an oligonucleotide primer. We utilized TdT's ability to incorporate non-natural fluorophore dNTPs into a long polymer chain of single stranded DNA (ssDNA). We quantified the TdT mediated signal amplification on the surface by immobilizing ssDNA oligomers on a glass surface followed by surface initiated enzymatic polymerization of DNA. We examined the effect of the concentration of the different natural dNTPs, and the molar ratio of fluorescent dNTPs to natural dNTPs on the length of the polymerized DNA strands. We optimized the reaction conditions and found that a large number of fluorescent nucleotides (up to ~100 fluorescent dNTPs) as template for TdT are necessary to initiate the polymerization conditions to incorporate a large number of fluorescent nucleotides (up to ~100 fluorescent dNTPs) into the ssDNA chain catalyzed by TdT. For Cy3-labeled dATP, this translated to a maximum of ~40 fold signal amplification through the incorporation of multiple fluorophores into the extended DNA chain. This methodology has the potential to both amplify the fluorescence signals and to provide a direct measurement of the amino acid composition.


Surface patterning is often used to immobilize biomolecules including biotinylated, oligonucleotides and small ligands, to localise surface reactions for bioassays and to provide desired cell and bacterial adhesion. This study reports extensive surface analysis of a commercial PEG-based surface chemistry with active ester (NHS)-activity in patterned films. The study followed sequential immobilization and masking reactions on photolithographic patterns used to immobilize peptides, proteins, and cultured cells to specific patterned regions of NHS-activated or de-activated chemistry. [1] Biotin and peptide patterns were correlated to patterned reactive NHS surface chemistry using high-resolution time-of-flight secondary ion mass spectrometry (ToF-SIMS) for each species. Cell growth and patternning in 15-day serum cultures followed peptide patterns. In other patterned samples, mixed protein (streptavidin and HaloTag [2]) solutions produced specific - self-recognized - binding patterns on photolithographically patterned affinity ligands for each (i.e. biotin and chloroaluminate, respectively). The approach uses high-affinity protein-surface self-selection onto patterned PEG-NHS surfaces that exhibit intrinsically low non-specific adsorption background. Fluorescence images and ToF-SIMS imaging of the resulting protein surface selection from mixtures support highly specific interactions of proteins with their respective ligands patterned on the surface. [2] On-going work comparing mixtures support highly specific interactions of proteins with their respective ligands patterned on the surface. A sandwich assay or the target strand in a DNA microarray provides the 3'-OH groups necessary to initiate on-chip fluorescence amplification of the binding event.

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

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

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

Though surface coatings of Poly-Ethylene Glycol (PEG) has been 
recognised for decades as a particularly effective non-fouling surface, recent 
advances in polymer brush fabricated thin film Poly-Oligo(Ethylene 
Glycol) Methyl Methacrylate (POEGMA) has presented a myriad of novel 
applications. The capability to easily tune brush height and still maintain a 
high surface grafting density has been shown to prepare surfaces which 
especially eliminate the non-specific adsorption of both proteins and cells.

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

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

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

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

**BI-ThP16** Template-stripped PZT Thin Films as Substrates for Charge 
Assisted Assembly of Biological and Organic Molecules. *R.E. 

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

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

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

Using natural compounds such as flavonoids in various diseases are under 
special consideration. Unfortunately poor solubility of flavonoids is an 
important limitation in preparing pharmaceutical dosage forms. It is 
reported that preparing nanoparticles can overcome poor solubility problem 
of the mentioned compounds. The O/W nano dispersions are a group of 
preparations in which the poorly soluble drugs could be dissolved in the oil 
core and adsorbed on the O/W interface. Dispersion of the interface was 
prepared by solvent diffusion method in organic phase and lecithine. After 
adding the dispersion to the aqueous phase, ultrasoundation and ultra 
centrifugation were carried out. A mixture of tween 80 and water were 
added to the mixture and then ultrasonicated again. Solvent was removed 
under reduced pressure at 50 C using nitrogen-purged vials. The particles 
were then evaluated for size and size distribution, zeta potential ( ZEN 
5000, Malvern, England ), shape, percent entrapment, and in vitro 
dissolution.

Differential scanning calorimetry method was used to understand the 
thermotropic properties and phase transition behavior. Droplet size 
nanoparticles of ferulic acid was 108 ± 12 nm.

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

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

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

Interfacing living matter to electronics with the ability to monitor and 
deliver spatio-temporal signals to cells or cell networks is promising for 
various fundamental biophysical studies and also for applications such as 
high resolution neural prosthetics, on-chip electrically addressed artificial 
neural networks and arrayed on chip patch-clamps. Developing an inorganic 
nanostructure that can specifically and non-destructively incorporate into 
biological membranes is the key to such an interface. We report an 
approach towards this interface by functionalizing a nanoscale metallic post 
to mimic a transmembrane protein to directly insert into the lipid membrane 
and form a tight seal. These post-electrodes were formed by vaporization 
and lift-off onto conductive bottom electrodes, with 5-10 nm thick hydrophobic bands around the edge of the post formed by molecular self assembly. 
We recently reported AFM measurements of these posts inserting into lipid bilayers and showed that different molecular functionalizations adhered within the hydrophobic lipid core with different strengths 
depending on their molecular mobility. Here we describe nanoscale 
electrical measurements with these post-electrodes on red blood cells to 
determine the leakage current at the electrode-membrane interface.
BI-ThP20 An Alternative Solution Based Approach to PTCDI-Melamine Network Fabrication on Au(111). V. Korolkov, N. Haggerty, M. Blunt, S. Allen, C.J. Roberts, S.J.B. Tendler, University of Nottingham, UK

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

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

Optimal conditions for oligonucleotide adsorption into the network-pores were also determined. This work employed several complementary surface analytical techniques to image the network structure (STM, AFM) and the controlled deposition of biomolecules (AFM, XPS).
Biomaterial Interfaces
Room: K - Session BI+AS+-NS-FrM

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

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

Patterning of many types of biomolecules over length scales ranging from micrometers to nanometers is of great interest for biosensors, cell culture dishes, medical implants and tissue engineering. Ideally these devices require attachment of biomolecules at specific locations on solid substrates with precisely controlled chemistry, but to function fully the non-specific adsorption in surrounding regions must be prevented. Currently, the most widely used techniques for patterning are photolithography, soft lithography and electron beam lithography, all of which involve multi-step surface modification directly onto substrates, and are time consuming and expensive. We have shown recently that highly ordered binary colloid patterns, with controllable dimensions, can be generated from simple self-assembly of large and small particles onto surfaces, where single layers of large particles are surrounded by crystals of smaller particles. In addition, when the particles are pre-coated with proteins (e.g. albumin, lysozyme and antibodies) the assembly process also takes place. This opens up the possibility of patterning many proteins on one substrate with controllable dimensions and high order. The crystals are also used to generate chemical patterns since the large particles act as a mask during, for example, sputtering of Au, since the region in contact with the substrate is uncoated. The thickness of gold features can be controlled by the sputtering time. We demonstrate that the resultant Au layer can be coated with a protein resistant mercapto-o1go(ethylene glycol) layer (1-(mercapto-11-undecyl)-tri(ethylene glycol)) that allows selective adsorption of fluorescently labelled proteins, such as FITC-labelled antibodies or rhodamine-labelled albumin, only onto the SI regions of the pattern. In another approach, binary patterns made from silica and amine polystyrene particles are heated at 100 °C (above glass transition temperature of polystyrene) followed by etching with HF to remove the silica particles creating highly ordered 2- and 3D porous substrates. In summary, we introduce a novel method for generating highly-ordered patterns from colloid crystals that is very fast, inexpensive, and allows patterns of multiple biomolecules over large areas in 2- and 3D.

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

Nano-patterned arrays of biomolecules are a powerful tool to address fundamental issues in many areas of biology. Combining nano lithography and biomolecular self-assembly strategies, we report on the fabrication of nanopatterned biomimetic surfaces and their use in a variety of biological studies.

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

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

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

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

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

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

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

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

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

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

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

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

We have spatially selectively deposited a zwitterion compound from the class of N-alkylaminorosorcinolines (or 4,6-bis-dialkylaminobenzoic-1,3-diones, C₆H₂(NHR)₂(O)₂), compounds, where R =C₅H₁₁. These molecules have large long local dipolar moments, which arise from delocalized π electrons. The zwitterion “core” loses aromatic character due to the large charge separation. This charge separation provides this type of zwitterion molecule with a large electric dipole moment across the “benzene” like plane. Unlike the ferroelectric materials, the electric dipole of this class of zwitterions
when adsorbed on metal surface (and most substrates) is not switchable, which makes these zwitterion compounds more like an electret. We have been able to demonstrate that at least one of this class of zwitterion compound will selective adsorb from solution on periodically poled lithium niobate substrates using infra-red spectra-microscopy. The spatial localization zwitterion on lithium niobate suggests that the ferroelectric poling of lithium niobate either alters the surface chemistry of lithium niobate or that there is some dipole-dipole interaction between the substrate and the zwitterion. We believe the interaction is an interface effect as no alteration in the bulk properties has been observed from spatially resolved near edge X-ray adsorption fine structure (NEXAFS) of the bulk properties. The spatial zwitterion structure is consistent with the periodically poled lithium niobate structure. Crystals of periodically poled lithium niobate (PPLN) with congruent composition (Crystal Technologies) were used as deposition templates. A periodic domain structure (period of ~28 µm) was fabricated by depositing a photoresist mask on the +c sample face and by applying a voltage of 10 kV through a fixture with an electrolyte solution. The mask was removed after poling by means of chemical-mechanical polishing leaving behind a bare ferroelectric surface, prior to the exposure to the zwitterion molecular solution.
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