Wednesday Morning, October 17, 2007

Biomaterial Interfaces

Room: 609 - Session BI-WeM

Nano-Engineered Biointerfaces

Moderator: A. Chilkoti, Duke University

8:00am **BI-WeM1 Multifunctional Quantum Dots for Biomedical Detection and Imaging**, ***X. Gao***, University of Washington **INVITED**

Metal and semiconductor nanoparticles in the 1-50 nm size range are of considerable current interest, not only because of their unique size-dependent properties but also their dimensional similarities with biological macromolecules (e.g., nucleic acids and proteins). These similarities could allow an integration of nanotechnology and biology, leading to major advances in medical diagnostics, prognostics, and targeted therapeutics. In this talk, I present recent development of multifunctional nanostructures for biomedical applications, such as bioconjugated nanoparticles for in vitro ultrasensitive detections and in vivo molecular imaging.

8:40am **BI-WeM3 Lateral Bilayer Fusion to Nanofunctional Probes**, *B.D. Almquist,* ***N.A. Melosh***, Stanford University

The cell membrane is one of the most vital components of a cell, and crossing through this barrier is a crucial component of biotechnology. Integration of inorganic structures with the cell membrane is poorly understood, and current techniques involve creating holes in or puncturing cell membranes to control access into the cell. However, functionalized materials with nanoscale hybrophobic layers may be able to directly fuse the lipid membrane edge to an inorganic structure, enabling non-disruptive electrical and chemical access into the cell. We have tested whether nanoscale inorganic probes integrate into the hydrophobic core of a lipid bilayer using an AFM probe with hydrophobically functionalized bands 5-20 nm thick at the end of the tip. We quantitatively measure the adhesion strength between the probe and the lipid bilayer, and correlate this molecule-membrane force with the molecular structure. We find the thickness of the nanoscale band and the identity of the hydrophobic molecules alters the ability to fuse to the membrane.

9:00am **BI-WeM4 GaN Nanowires for DNA-Sensing Applications**, *C.P. Chen, A. Ganguly, C.H. Wang,* ***L.-C. Chen***, National Taiwan University, *C.W. Hsu, Y.-K. Hsu, K.-H. Chen*, Academia Sinica, Taiwan

A novel DNA-sensing system based on GaN nanowires (NWs) is presented coupled with their electrochemical impedance and photoluminescence measurements. GaN is well established now for a variety of optoelectronic applications. However, while its inherent bio-compatibility has also been recognized, its application as bio-sensors has been surprisingly lacking till date. Meanwhile, one-dimensional nanostructures have attracted huge interest as potential building blocks for the future nanoelectronic devices. In this report, GaN NWs are used as a transducer for DNA-sensors, by immobilizing single-strand DNA (ssDNA) molecules through covalent binding using organosilane linker (MPTS). The MPTS-modified GaN NWs exhibited an electrochemical window remarkably wider than those of boron-doped diamond or carbon nanotubes reported to date. The immobilization of ssDNA and the subsequent hybridization to double-strand DNA (dsDNA) were confirmed using confocal microscope. Electrochemical impedance measurement showed that interfacial electron-transfer resistance (Ret), from solution to transducer surface, increased significantly when pristine GaN NWs were immobilized with ssDNA, along with a formation of additional semicircle region at lower frequency in Nyquist plot. The unique appearance of double-semicircle region for ssDNA-immobilized NWs, compared to single-semicircle region for pristine GaN NWs, leads to the idea of formation of double-capacitance layer in series. The phenomenon is more prominent by the appearance of double peaks in the plot of phase angle vs. frequency (Bode plot), the second peak, formed after ssDNA-immobilization, showed further increase under the hybridization to dsDNA, and consequently reduces the overall impedance. Moreover, quenching behavior in photoluminescence of the GaN NWs was distinguishable for the ones immobilized with ssDNA and the same hybridized to dsDNA. Both the technique implies the ability of oligonucleotides, immobilized on the NW-surface, to interact with other biomolecules. The dual and label-free sensing capability in impedance and photoluminescence of GaN NWs makes them effective DNA transducers.

9:20am **BI-WeM5 Functional Protein Chip Nano-Templates by Chemical Lithography and Multivalent Chelator Techniques**, ***A. Turchanin***, *M. El-Desawy, M. Schnietz, A. Gölzhäuser,* University of Bielefeld, Germany, *A. Tinazli, H. Großmann, R. Tampé,* Johann Wolfgang Goethe-University, Germany, *H.H. Solak,* Paul Scherrer Institut, Switzerland

To study protein function and interaction, there is a high demand for specific, stable, highly parallel, and functional protein arrays on solid substrates. To implement this, we propose a protein chip approach based on the combination of electron induced chemical lithography[1] with aromatic self-assembled monolayers (SAMs) and multivalent chelators[2] for high-affinity capturing of His-tagged proteins. Templates for functional and switchable His-tagged protein architectures were created by electron induced changes in 4'-nitro-1,1'-biphenyl-4-thiol SAMs on gold (NBPT). Chemical nanopatterns were generated in SAMs by both electron beam lithography (EBL) and extreme UV interference lithography (EUV-IL). As a model system carboxy derivative tris-NTA chelators were studied. Different steps of the protein chip assembly (fabrication of amino terminated cross-linked areas, generation of protein repellent EG3-OH thiols matrix, grafting of tris-NTA chelators, and chelating efficiency of Ni(II) ions) were characterized in detail by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). The functionality of the generated protein chips was shown in situ, under physiological conditions by AFM and scanning fluorescence microscopy measurements via specific, homogeneous, oriented and reversible immobilization of His_6-tagged 20S proteasome and fluorescence labelled His_{10}-tagged maltose binding proteins (MBP). We will present highly parallel large area (~10 mm^2) protein arrays with the lateral dimensions of periodic features ranging from 1000 nm to 50 nm.

[1]A. Gölzhäuser, W. Eck, W. Geyer, V. Stadler, T.Weimann, P. Hinze, M. Grunze, Adv. Mat. 13 (2001) 806

[2]A. Tinazli, J. Tang, R.Valiokas, S. Picuric, S. Lata, J. Piehler, B. Liedberg, R. Tampé, Chemistry 11 (2005) 5249.

9:40am **BI-WeM6 Protein Patterning by Scanning Near-Field Photolithography**, ***G.J. Leggett***, *R.E. Ducker, M. Montague, K.S.L. Chong,* University of Sheffield, UK

Photolithography is a convenient and rapid route to the fabrication of patterned self-assembled monolayers for the control of biological organisation. Alkanethiols may be photo-oxidised by exposure to light with a wavelength of 244 nm to yield soluble sulfonates which may be displaced by complementary thiols to yield clean, well-defined chemical patterns. Protein patterning is complicated by the problems of non-specific adsorption. Most proteins adhere to most surfaces, rendering protein patterning difficult. Oligo(ethylene glycol) (OEG) terminated self-assembled monolayers are attractive because they resist protein adsorption very effectively. Here we have explored the possibility of patterning OEG-terminated SAMs using photolithography. Rates of photo-oxidation of OEG-terminated thiols have been measured using contact angle goniometry, static SIMS and friction force microscopy. The kinetics observed appear to be different from those observed for other thiols. The mechanism appears to be complex. Over longer exposures, photo-oxidation of the head group occurs. Subsequent patterning through a mask yields well-defined structures that consist of either methyl or carboxylic acid terminated thiols in regions exposed to UV light, to which proteins may, respectively, be adsorbed or covalently bound, and protein-resistant OEG-terminated adsorbates over the rest of the surface. Exposure using a near-field scanning optical microscope (scanning near-field photolithography, SNP) yields nanometre scale structures. At short exposures, photodegradation of the OEG chain occurs leading to the formation of aldehyde groups that covalently bind proteins with high efficiency. This provides a very convenient single-step route to the introduction of a reactive functional group, in a spatially selective fashion, to a protein resistant OEG monolayer. The amount of streptavidin bound to such a photo-modified monolayer is nearly as great as that bound using well-established carbodiimide-based methods on carboxylic acid terminated monolayers. Significantly, the photodegradation of the OEG terminal groups in this process is much more rapid than the oxidation of the thiol head-group and is, moreover, capable of excitation at longer wavelengths, where photo-oxidation of the thiol sulfur atom is no longer a possibility. Photopatterning thus appears to be a simple and versatile route to protein patterning.

10:40am **BI-WeM9 Multicomponent Nanoparticles for Controlled Intracellular Delivery to Targeted Cells**, *J.M. Bergen, I.K. Park, E. Kwon,* ***S.H. Pun***, University of Washington **INVITED**

Gene therapy offers the possibility of treating diseases by altering the protein expression profiles of affected cells. Nucleic acids can be condensed

by complexation with cationic polymers to nanoparticle structures called "polyplexes". These synthetic vehicles can be used to accomplish gene delivery but are usually limited in their applications due to poor delivery efficiencies in non-dividing cells. I will describe our work on developing multicomponent polyplexes that incorporate biologically-derived peptides that facilitate delivery. Peptides that mediate neuron-specific uptake and endosomal release were incorporated into polyplexes. The intracellular trafficking of these materials was also investigated by live cell imaging in compartmented chambers. Incorporation of functional peptides improves delivery efficiency to cultured, neuron-like PC-12 cells.

11:20am **BI-WeM11 Femtosecond Laser Ablation to Create Nanometer-Scaled Cell Adhesion Ligand Patterns**, ***R.C. Schmidt***, UC Berkeley/UC San Francisco Joint Graduate Group in Bioengineering, *D.H. Hwang*, *C.P. Grigoropoulos*, UC Berkeley, *K.E. Healy*, UC Berkeley/UC San Francisco Joint Graduate Group in Bioengineering

The goal of our project is to fabricate interfaces for mammalian cell culture that control cell fate via the spatial distribution of the individual focal adhesions cells use to interrogate the interface. To create nano-scale cell adhesion sites on a surface, a thin protein adsorption resistant polyethylene glycol (PEG) brush layer was synthesized via surface initiated atom transfer radical polymerization (SI-ATRP). The surface chemistry was verified with XPS, showing strong oxygen and carbon peaks consistent with a PEG film, and thickness of the dry film in air was calculated to be 10nm using a quartz crystal microbalance with dissipation (QCMD). The film was selectively ablated using focused femtosecond laser pulses, exposing the underlying quartz substrate as centers for adsorption or grafting of cell-adhesive molecules. Preliminary results at a wavelength of 400nm with a 50X objective demonstrated spatial resolution approaching 200nm based on atomic force microscopy (AFM) scanning of the ablated features. The practical resolution limit can be further improved (~10-100nm) by utilizing higher magnification lenses at shorter wavelengths or processing in the optical near-field. This technique allows us to generate arbitrary nanoscale protein patterns on the benchtop without specialized processing environments. These nanostructured surfaces will eventually allow us to decouple the effects of cell size and shape, focal adhesion placement, and ligand density on cell fate decision by directly controlling the number and area of focal adhesion complexes formed. Each variable can be modulated independently to determine the effects on cellular function and fate determination.

11:40am **BI-WeM12 Neuron Pathfinding on Functionalized Patterned, Gradient, and Fiber Biomaterial Surfaces**, *W.M. Theilacker*, *M.E. Boggs*, *S.K. Mbugua*, *S.P. Sullivan*, Univ. of Delaware, *D.E. Willis*, Nemours Biomedical Res. of Alfred I duPont Hospital for Children, *K.W. Dabney*, Alfred I duPont Hospital for Children, *J.L. Twiss*, Nemours Biomedical Res. of Alfred I duPont Hospital for Children, ***T.P. Beebe, Jr.***, Univ. of Delaware

This paper will present the recent results from a collaborative study that is aimed at developing novel growth-promoting substrates for injured and damaged neurons, with an emphasis on understanding the mechanisms of substrate-neuron interactions and the resulting modulation of intra-axonal signal transduction. Axons regenerating in vivo must traverse from a permissive into a non-permissive environment. We use the permissive environment of novel surface-grafted 2-D and 3-D materials to increase the capacity for axons to traverse into a non-permissive growth environment. We have generated novel patterned and well characterized 2-D and 3-D biomaterial growth substrates that mimic the environment encountered by the regenerating axons in the injured spinal cord. This is accomplished by engineered patterns and gradients with tailored composition of growth-promoting extracellular matrix molecules. We will also describe the results of studies aimed to observe and mimic how permissive growth substrates directly modulate axonal-substrate interactions and intra-axonal signal transduction. The program incorporates elements of surface chemistry, surface analysis, cell culture, optical microscopy, and neuroscience.

12:00pm **BI-WeM13 XPS, ToF-SIMS, NEXAFS and SPR Characterization of Nitrilotriacetic Acid-Terminated Self-Assembled Monolayers For Controllable Immobilization of Proteins**, ***F. Cheng***, *L.J. Gamble*, *D.G. Castner*, University of Washington

For immobilization of proteins onto surfaces in a specific and controlled manner it is important to start with a well-defined surface that contains specific binding sites surrounded by a nonfouling background. For immobilizing histidine-tagged (histagged) proteins, surfaces containing nitrilotriacetic acid (NTA) headgroups and oligo(ethylene glycol) (OEG) moieties are a widely used model system. The surface composition, structure and reactivity of mixed NTA/OEG self-assembled monolayers (SAMs) on Au substrates were characterized in detail using X-ray photoelectron spectroscopy (XPS), near-edge X-ray absorption fine structure spectroscopy (NEXAFS), time-of-flight secondary ion mass spectrometry (ToF-SIMS) and surface plasmon resonance (SPR) biosensing. XPS results for sequentially adsorbed NTA thiols followed by OEG thiols showed that OEG molecules were incorporated into a incompletely formed NTA monolayer until a complete mixed SAM was formed. Surface concentration of NTA headgroups was estimated to be 0.9 molecule / nm^2 from XPS results. Angle-resolved XPS and polarization-dependent NEXAFS results both indicated NTA headgroups were slightly reoriented toward an upright position upon OEG incorporation. Nitrogen-containing and OEG-related secondary ion fragments from the ToF-SIMS experiments confirmed the presence of NTA headgroups and OEG moieties in the monolayer. These fragments along with secondary ion amino acid fragments are being used to investigate the orientation and conformation of histagged proteins immobilized onto the NTA/OEG SAMs. SPR measurements of a histagged, humanized anti-lysozyme variable fragment (HuLys Fv) immobilized onto Ni(II) -treated mixed NTA/OEG SAMs demonstrated the reversible, site-specific immobilization of histagged HuLys Fv (170 ± 7 ng / cm^2) with strong binding affinity (approximately 43 nM). The mixed NTA/OEG SAMs without Ni(II) treatment exhibited good resistance to nonspecific adsorption of histagged HuLys Fv (< 2 ng / cm^2).

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