# Thursday Afternoon Poster Sessions

## Biomaterial Interfaces Room: Hall 3 - Session BI-ThP

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

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

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

#### BI-ThP2 Aqueous Polymer Nanografting: AFM patterning of Poly-L-Lysine on Oxide Surfaces, B.S. Davis, H.J. Conley, J.L. Knoebel, K.B.

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

#### BI-ThP3 High Sensitivity Electrochemcial Immunosensor Based on Plasma Modified TiO<sub>2</sub>/Chitosan, *R. Khan*, North East Institute of Science & Technology, India, *M. Dhayal*, Centre for Cellular and Molecular Biology, India

RF plasma treated nanocrystalline hybrid chitosan/TiO<sub>2</sub> matrix was used to develop high sensitivity electrochemical immnunosensor. The observed interfacial charge transfer resistance ( $R_{CT}$ ) and double layer capacitance ( $C_d$ ) of plasma modified matrixes were decreased. An improved strength of amide I and II groups in FTIR spectra were observed which was associated with enhancement in immobilized rabbit antibodies (IgGs) on plasma altered surfaces. Electrochemically q uantitative detection of *Ochratoxin-A* (OTA) concentrations varying in buffer solution was carried out on IgGs immobilized plasma treated and untreated ITO/CS/TiO<sub>2</sub> electrodes. Plasma modified electrodes had showed very good sensitivity at very low OTA concentrations whereas the untreated electrodes sensitivity was deprived.

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

We have studied the Fe 2p X-ray magnetic circular dichroism (XMCD) of individual magnetosomes - biomineralized ferrimagnetic nano-crystals in magnetotactic bacteria (MTB ) - using scanning transmission X-ray microscopy(STXM). Magnetosomes are intracellular magnetite (Fe<sub>3</sub>O<sub>4</sub>) or greigite (Fe<sub>3</sub>S<sub>4</sub>) nano-crystals (typically 30-60 nm in size), enclosed in a lipid membrane. A chain of magnetosomes is used by MTBs to orient relative to the earth's field, and guide motion to optimal living environments. Our initial goal, which has been achieved, was to demonstrate that the STXM has the capability to investigate magnetic properties of sub-50 nm areas in biological systems. The Fe 2p XMCD of individual Fe<sub>3</sub>O<sub>4</sub> magnetosomes of MV-1, a marine vibrio species of magnetotactic bacteria, was measured with the sample at 30 degrees relative to the beam to sense the in-plane magnetic component. This is the first such measurement of the XMCD of a single magnetosome to our knowledge. Evidence for multiple domains was found in some magnetosomes. In addition we have begun to explore the associated biochemistry by STXM

spectromicroscopy at high spatial resolution (30 nm) at the C 1s and O 1s absorption edges. The combined XMCD and biochemical imaging will help further the understanding of biomineralization processes present in MTB and other environmental organisms.

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

# **BI-ThP6** A Novel Technique for the Determination of Orientational Effects in Bio-Composite Materials, D. Ziskind, T. Geron, S. Fleischer, K.

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

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

Self-assembled Monolayers (SAMs), especially alkanethiols on gold, have been extensively used as model system for studying surface modification strategies. In this work, we utilize this platform to fabricate carbohydratemodified 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 carbohydratebinding proteins (lectins). XPS and ToF-SIMS results give a clear indication that the composition of mannose on the surface is directly proportional to its molar ratio in solution. However, when these sensors are exposed to lectins, XPS shows that the amount of protein is inversely proportional to the amount of mannose present on the surface. We performed SPR studies to obtain a quantitative comparison of the amount and multivalent binding of lectins on these mixed SAMs. Detailed study of this system using XPS, ToF-SIMS, SPR and ELLA suggests that an optimum density of mannose on the surface is required to improve the sensitivity and stability of these sensors.

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

Self-assembled monolayers (SAMs) have proved to be highly versatile systems for molecular patterning. Previous work using near-field optical methods has demonstrated that exceptional spatial resolution may be achieved using SAM resists, by exploiting the fact that the photosensitive group is confined to a monomolecular film adsorbed to a solid surface. However, near-field methods are serial in nature. Here we have exploited interferometric approaches in combination with SAM resists to fabricate a variety of nanostructured materials. Extremely high resolution has been achieved by exploiting the monolayer nature of the resist. Interferometric lithography (IL) is rapid, and uses minimal instrumentation. IL using a cw, 244 nm frequency doubled Ar-ion laser source has been found to yield structures as small as 35 nm using SAM resists, over macroscopically extended areas. SAMs of alkanethiols on gold may be photo-oxidized to yield weakly bound sulfonates that may be displaced by solution-phase adsorbates to yield patterns of chemical composition. Here, we demonstrate the fabrication of patterns of surface free energy with a period of 200 nm. Protein adsorption may be controlled by using IL to selectively photodegrade oligo(ethylene oxide) (OEG) terminated SAMs of alkanethiols on gold and of trichlorosiloxanes on glass. Nanopatterned streptavidin formed this way retains its ability to bind biotinylated proteins. Finally, monolayers of phosphonic acids on titanium dioxide may be readily patterned and used as templates for the fabrication of a variety of architectures, including 35 nm TiO2 structures on glass. IL is an inexpensive, fast and convenient means of producing molecular nanostructures over square centimetre and larger areas.

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

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

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

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

[1] R. Dahint, E. Trileva, H. Acunman, U. Konrad, M. Zimmer, V. Stadler, M. Himmelhaus, Biosens. Bioelectron., 22, 3174-3181 (2007)

[2] P. Buecker, E. Trileva, M. Himmelhaus, R. Dahint, Langmuir, 24, 8229-8239 (2008)

[3] V. Stadler, T. Felgenhauer, M. Beyer, S. Fernandez, K. Leibe, S. Güttler, M. Gröning, K. König, G. Torralba, M. Hausmann, V. Lindenstruth, A. Nesterov, I. Block, R. Pipkorn, A. Poustka, F. R. Bischoff, F. Breitling, Angew. Chem. Int. Ed., 47, 7132-7135 (2008)

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

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

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

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

#### References:

<sup>[1]</sup>Takahashi, H., Emoto, K., Dubey, M., Castner, D. G., Grainger, D. W., *Adv. Func. Matls.* 2008. **18**(14): p.2079-2088.

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

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

BI-ThP14Temperature-InducedConformationalChangesofAntifreezeProteins inAqueousSolutionviaOverlayer-EnhancedAttentuatedTotalReflectanceFourierTransformInfraredSpectroscopy(OE-ATR-FTIR),T.Ng,D.P.Land,UniversityofCalifornia, Davis, X.Wen,California State University,LosAngeles

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 antifreze protein extracted from *Dendroides canadensis* is determined in the aqueous and frozen states and

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

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

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

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

Traditionally, EFN has been utilized to produce oxide-rich regions available for further reaction sites processing. Integration of a responsive, nonfouling, 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, filmthickness dependent, threshold bias voltage is reported. Films have been characterized using Xray Photonelectron Spectroscopy, Atomic Force Microscopy, and Contact angle measurements.

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

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

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

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

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

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

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

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

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

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

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

Two dimensional (2D)-controlled adsorption is a versatile tool for creating well-defined arrays of biological molecules on surfaces. Such surfaces hold potential for a wide range of future applications, including for the development of biosensors and biomolecular screening technologies. Functionalizing a surface with some periodical structure (or a network) is one promising way to spatially control the adsorption process. Hydrogenbonded 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).

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— N — Ng, T.: BI-ThP14, 2 Nguyen, H.: BI-ThP8, 1 - 0 -Obst, M.: BI-ThP4, 1 – P – Pourmorad, F.: BI-ThP17, 3 — R — Ratner, D.: BI-ThP8, 1 Roberts, C.J.: BI-ThP20, 4 – Т — Takahashi, H.: BI-ThP13, 2 Tendler, S.J.B .: BI-ThP20, 4 Tizazu, G.: BI-ThP9, 1 Tjong, V.: BI-ThP12, 2 - V -Verma, P.: BI-ThP19, 3 – W – Wagner, H.D.: BI-ThP6, 1 Waly, N.: BI-ThP11, 2 Wang, H.: BI-ThP1, 1 Wang, X .: BI-ThP1, 1 Wen, X.: BI-ThP14, 2 – Y — Yan, M.: BI-ThP1, 1 Yellen, B.B.: BI-ThP16, 3 Yu, H.: BI-ThP12, 2 — Z – Zauscher, S.: BI-ThP16, 3 Zhang, K.: BI-ThP6, 1 Ziskind, D.: BI-ThP6, 1