## Biomaterial Interfaces
## Room 307 - Session BI-MoM
### Protein-Surface Interactions
**Moderator:** A. Chilkoti, Duke University

8:20am **BI-MoM1 Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) and Surface Plasmon Resonance (SPR) Determination of Surface Bound Anti-Lysozyme Orientation***, **N. Xia**, N.T. Samuel, P.S. Stayton, D.G. Castner,* University of Washington

Static time-of-flight secondary ion mass spectroscopy (ToF-SIMS) and surface plasmon resonance (SPR) were used to analyze the orientation of immobilized proteins. A model protein with a well-define structure, the Fv fragment of a humanized anti-lysozyme (HuLys), was used. A His-tag linked to the C-terminal of the heavy chain was located opposite to the lysozyme binding domain (complementarity-determining region, CDR). We immobilized nitrilotriacetic acid (NTA) onto a self-assembled monolayer (SAM) of oligo(ethylene glycol)-terminated (OEG) thiol on Au. OEG provides a low-fouling background and NTA binds nickel ions, leaving two coordination sites available for interaction with the His-tag of HuLys. SPR experiments showed after nickel activation the NTA/OEG surface specifically bound HuLys Fv via the His-tag and that the immobilized HuLys Fv had nearly full antigen (lysozyme) binding capacity, suggesting a uniform CDR-exposed orientation of HuLys Fv on the surface. For comparison, we also activated the OEG SAM with carbonyldiimidazole (CDI), which binds protein via amine/imidazolyl carbamate chemistry and should result in a random protein orientation. This was supported by the SPR results, which showed that only ~50% of HuLys Fv immobilized on the CDI/OEG surface had antigen binding capability. The ToF-SIMS spectra of HuLys Fv immobilized on NTA/OEG and CDI/OEG surfaces were then compared. It was found that the peaks at m/z=81, 82 and 110 had lower intensities in the spectra of HuLys Fv immobilized on the NTA/OEG surface. These peaks all correspond to the primary mass fragments from the amino acid histidine. Since wild-type Hulys Fv has only 2 histidine residues, the ToF-SIMS results confirmed that HuLys Fv was immobilized onto the NTA/OEG substrate via the His-tag, which should be located at the bottom of the protein layer.

8:40am **BI-MoM2 Identification of Proteins in the Presence of Topographic Features using TOF-SIMS***, **S. Rangarajan**, B.J. Tyler,* University of Utah

In an earlier study, we had demonstrated the use of statistical modeling using mixture models and Principal Components Analysis (PCA) in characterizing samples having a relatively simple chemistry and coupled with topographical features. This study involves a very important extension to analysis of samples with more complex chemistry, such as with proteins. ToF-SIMS has been extensively used to characterize proteins, in spite of the inherent difficulties associated with spectral interpretation.@footnote 1,2@ Multivariate methods have also proved to be invaluable in the discrimination of adsorbed proteins on flat substrates.@footnote 3@ In this study, Polystyrene micro-spheres were adsorbed with different proteins and statistical models as well as discriminant techniques such as PCA were used to analyze the ToF-SIMS images. Discrimination of protein spectra from the images was then performed using the afore mentioned techniques, without a priori information about the type of protein adsorbed onto the sphere surfaces. Some of the models, such as the multinomial mixture model were found to yield more information than previously thought. References. @FootnoteText@ @footnote 1@ M.S.Wagner and D. G. Castner, Langmuir 17, 4649 (2001). @footnote 2@ D. S. Mantus and B. D.Ratner, Analytical Chemistry 65, 1431 (1993).@footnote 3@ M.S.Wagner, B.J.Tyler, and D. G. Castner, Analytical Chemistry 74, 1824 (2002).

9:00am **BI-MoM3 Immobilized Microarrays of Capture Agents for Bioassay: A Return to the Past for Protein Surface Stability?***, **D.W. Grainger**, P. Gong,* Colorado State University*; M. Lochhead, S. Metzger,* Accelr8 Technology Corporation **INVITED**

Microarrays of antibodies, nucleic acids, and antigens all encounter problems with prolonged bioactivity and desired capture sensitivity in immobilized formats. Surface chemistry is used to produce high target capture activity (high signal sensitivity) with low non-specific binding (noise). These surfaces can exhibit shelf-life problems limited, for example, by intrinsic hydrolysis of amine-reactive coupling chemistry (active esters, aldehydes) even under protective conditions. Reactive commercial array surfaces targeting amine-reactive nucleic acids or proteins have been regenerated in situ using N-hydroxysuccinimide to re-activate amine reactivity, improving functionalization of the commercial surfaces and improving immobilization of amine-terminated probes above original capacity. XPS and ToF-SIMS results for surface re-derivatization are correlated with DNA probe immobilization and target capture efficiencies. In a second effort, contact printed immobilized antibodies against a probe analyte on commercial polymer microarraying surfaces (70-micron spots) were assayed for model target capture (goat IgG) in sandwich immunoassay with fluorescently labeled secondary antibodies in full goat serum, imaged by fluorescence scanning. Off-array noise and on-array signal were compared as a function of printed antibody concentration. Despite masking with prescribed protocols (e.g., BSA or polymer masking), assay signal:noise was markedly improved on a non-masked three-dimensional polymer hydrogel commercial chemistry. Last, commercial arraying surfaces were used to exploit nucleic acid amplification (PCR reaction)on-array, capture fluorescently labeled target amplicons with printed probes, and rinse away all PCR reaction reagents in a single-step assay without prior separations or compromise to signal:noise performance. This provides substantial advantages in time and effort should sufficient signal:noise be achieved without costly, tedious PCR separation steps.

9:40am **BI-MoM5 Limitations of Molecular Streptavidin/Anti-biotin Antibody Architectures using Micro-contact Printed Biotinylated Thiols***, **Ch. Grunwald**,* Ruhr-University Bochum, Germany*; N. Opitz,* Max-Planck Institute for Molecular Physiology, Germany*; S. Herrwerth, W. Eck,* University of Heidelberg, Germany*; J. Kuhlmann,* Max-Planck Institute for Molecular Physiology, Germany*; Ch. Woell,* Ruhr-University of Bochum, Germany

Atomic force microscopy (AFM) and confocal fluorescence microscopy have been used to study the interaction of streptavidin and anti-biotin antibodies with a patterned, biotinylated organic surface. This system presently attracts considerable interest because of its potential for molecular architectures employing protein-protein interactions. The substrates were prepared by first using the µCP technique to print a periodic pattern of an oligoethylenglycol (OEG) self-assembled monolayer (SAM) on clean gold surfaces. The pattern consists of squares (40 µm x 40 µm) which are separated from each other in each direction by 10 µm. By immersing the stamped substrates into a mixture of OH-terminated and biotinylated organothiols a patterned SAM is obtained. These 2D-SAM patterns have been imaged via contact atomic force and lateral force microscopy as well as with tapping-mode AFM. The patterned SAMs were then incubated with two fluorescence-labelled proteins exhibiting a strong affinity towards biotin, streptavidin and anti-biotin antibody. Incubation time, temperature and concentration of the protein solution as well as the biotin surface concentration were varied systematically. The comparison of the AFM-data with the results of the fluorescence microscopy allows for important conclusion on the protein-protein binding, in particular concerning reproducibility, unspecific binding and protein resistance.

10:00am **BI-MoM6 A Comparison of Microcontact Printed and Solution Adsorbed Cytochrome c Protein Films on Indium Tin Oxide Electrodes***, **A. Runge**, S. Saavedra,* University of Arizona

The immobilization of proteins on a surface in a controlled way that retains their function is one of the challenges in making a functioning biosensor. Electrochemical biosensors use redox active proteins to impart selectivity to the electrode surface on which they are immobilized (either adsorbed or covalently attached). The orientation of proteins on the surface is presumed to be important for proper functioning of the device. We are investigating using microcontact printing as a way of immobilizing cytochrome c onto indium tin oxide electrodes in order to determine how the method of immobilization affects the orientation and function of the protein. We are presenting the results of three different methods for forming protein films on indium tin oxide that have been characterized and compared using cyclic voltammetry and X-ray photoelectron spectroscopy. Preliminary results from surface sensitive polarized spectroscopic studies will also be discussed. Protein films formed by adsorption of cytochrome c out of solution and by microcontact printing with both hydrophobic and hydrophilic PDMS are compared in terms of total and electrochemical surface coverage, standard reduction potential and rate of electron transfer with the ITO surface. Plasma treatment of the PDMS stamps, which makes them hydrophilic, dramatically increases the surface coverage of printed films to the level of solution adsorbed films. We have demonstrated that a redox active protein can be microcontact printed onto an electrode surface with its capability for direct electron transfer with the

surface intact. The total surface coverage of the different films were compared using X-ray photoelectron spectroscopy.

10:20am **BI-MoM7 Using Nanografting to Position with Predictable Orientation, De-novo Proteins on Gold***, **Y Hu**, M. Case, G. McLendon, T.K. Vanderlick,* Princeton University*; D. Vanderah,* National Institute of Standards and Technology*; B. Nickel,* Princeton University*; M. Mrksich,* University of Chicago*; G.Y. Liu,* University of California, Davis*; G. Scoles,* Princeton University

An extensive research effort has been trying to make biosensors at the nanometer scale, especially selective detection devices with molecular recognition sites. We have approached this problem from a unique angle by using nanografting, which is to use an AFM tip to remove thiol molecules from a designated area of a self-assembled monolayer (SAM) while different thiol molecules from a contacting solution will self-assemble onto the exposed gold sites. By incorporating cysteine residues at the end of the peptide chains of [Fe(V@suba@L@subd@C-long)@sub3@]@super2+@, the proteins acquire the possibility to stand vertically on a gold surface. By using nanografting, the proteins could be patterned into islands about tens of nm wide and their properties could be measured in a differential way using the surrounding SAM as a reference. The height of these islands measures 3.2nm±0.4nm which, added to the 2.2 nm of the C@sub18@ SAM, corresponds well with the model height of the proteins. Effectively maximizing the signal-to-noise ratio of biosensors depends also on the ability to prevent protein nonspecific surface adsorption. It has been found that SAMs of thiols containing short oligomers of the ethylene glycol group prevent the adsorption of most proteins under a wide range of conditions. However the mechanism has not been clearly explained. It is observed that both SH(CH@sub2@)@sub11@(EO)@sub3@-OH and SH(EO)@sub6@-(CH@sub2@)@sub17@-CH@sub3@ reversibly compressed to half of their height under small imaging forces(0-10nN) in ethanol or 2-butanol. When the force is increased to over ~50nN, irreversible compression happened. Moreover, when the solution is changed to water mixture, the SH(EO)@sub6@-(CH@sub2@)@sub17@-CH@sub3@ is found to decrease its height significantly, and become much less compressible. By offering a model to explain the compressibility changes observed, we hope to offer some insight into the protein resistant properties of PEG-containing layers.

10:40am **BI-MoM8 Nanoscale Control of ECM Proteins for Cell Adhesion***, H. Wang, L. Liu, **S. Chen**, T. Barker, H. Sage, B.D. Ratner, S. Jiang,* University of Washington

Osteopontin (OPN) is an important extracellular matrix protein shown to function in wound healing, inflammation and foreign body reaction and has been identified as a potential target for engineered biomaterials. The secreted protein acidic and rich in cystein (SPARC/osteonectin/BM-40) is associated with events characterized by changes in cell shape and mobility. In the work, we first report control of OPN orientation and conformation on charged self-assembled monolayers (SAMs) for cell adhesion. Our atomic force microscope (AFM) results show that the amount of adsorbed OPN on -COOH surface is slightly less than that on -NH2 surface. Results from in vitro cell adhesion assays show that on NH2 surface BAEC adhesion and spreading are more. By comparing these results, it is suggested that the orientation/conformation of OPN on -NH2 positively charged surface is more favorable for cell interactions than on -COOH negatively charged surface. Second, AFM is used to image the binding of OPN onto individual triple-helical collagen I monomer on freshly cleaved mica for the first time. We also use anti-OPN antibody to assist for better visualization. Analysis of AFM results clearly shows binding patterns of OPN to collagen I. Finally, the interactions of SPARC with ECM proteins, such as collagen I and fibronectin, are characterized and quantified using AFM and surface plasma resonance (SPR). Cell culture and adhesion assays are used to study SPARC as a modulator of the adhesive process of cells seeded on ECM proteins. The influence of SPARC-collagen I interaction is studied using smooth muscle cells while the influence of SPARC-fibronectin interaction is studied using endothelial cells.

11:00am **BI-MoM9 Activity of the Model Enzyme Urease Adsorbed on Different Colloidal Oxide Particles***, **K. Rezwan**, J. Voros, M. Textor, L.J. Gauckler,* ETH Zurich, Switzerland

Whilst metal oxides are directly used for applications where their extreme hardness is necessary (e.g. femoral head replacement), most metallic biomaterials are themselves covered by a protective, stable oxide film such as titanium oxide on titanium. In these cases proteins only interact with the oxide film and not with the underlying metal. Closer investigations of the protein - oxide interface are therefore vital to the biomaterials field as it strives to make the transition from merely bio-inert to fully bioactive implant materials. It is assumed, that not only the amount of adsorbed protein but also its conformation is important for cell proliferation. A change of protein structure would hamper the cell receptors (situated within the cell membrane) to recognize the specific protein function. As a consequence, the cell would not adhere and proliferate on the preliminary adsorbed protein layer and not accept the adsorbed proteins as body own proteins but rather see them as intruders. This fatal mistake, made by the cell, is assumed to be one of the reasons which promotes local inflammation and tissue mutations. Assessing the conformational changes of a protein after adsorption is a delicate matter and can be measured by using for instance circular dichroism. In the case of enzymes, one can also measure the activity of the enzyme before and after adsorption. We used the model enzyme urease for our studies where the catalysis of urea was monitored by measuring the electrical conductivity as a function of time. The aim of this study was to compare the activity (and hence the conformational state) of urease after adsorption onto different colloidal oxide particles. We found that urease adsorbed onto TiO2 showed the highest activity and urease on Al2O3 the lowest. The measurements showed also an adsorption time dependency, which indicated further conformational changes after adsorption.

11:20am **BI-MoM10 In situ Real-time Atomic Force Microscopy Studies of Lysozyme and RR02 Protein Crystal Growth at Surfaces***, T.R. Keel, S. Allen, M.C. Davies, C.J. Roberts, **S.J.B. Tendler**, P.M. Williams,* University of Nottingham, UK

The successful application of crystallography to fields such as structural biology and rational drug design has been largely due to the availability of single crystals of the macromolecule of interest. However, relatively little is understood about the fundamentals of macromolecular crystal growth. Here, we have utilized the technique of atomic force microscopy (AFM) to study protein growth at surfaces. Two different protein systems, at both the micro- and nanometre scale have been investigated. The first is a model system, lysozyme. We have investigated two polymorphs of the lysozyme crystal and present data concerning the effect of supersaturation (@sigma@) on the growth rates and mechanisms of growth of the crystals. Molecular resolution studies have also been carried out and the observed periodicities are in good agreement with the known unit cell dimensions. Also presented are preliminary results from a second protein system, RR02, which is a response regulator protein found in streptococcus pneumoniae. RR02 is relatively straightforward to crystallize, but unlike lysozyme, many of the grown crystals diffract poorly and x-ray studies yield little structural data. We have used the AFM to investigate these crystals and compare the results with the lysozyme studies.

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