Biomaterial Interfaces Group
Room 326 - Session BI-MoM

Protein Solid-Surface Interactions

Moderator: J.J. Hickman, The George Washington University

8:20am **BI-MoM1 Effect of Surface Rheology on Anti-Adhesive Properties of Water-Soluble Thin Films**, ***J.W. Schneider***, *W.R. Barger, J.-B.D. Green, R.F. Brady, Jr., G.U. Lee,* Naval Research Laboratory

Thin films of water-soluble polymers, including poly (ethylene oxide), have been used to improve the biofouling resistance and biocompatibility of surfaces. Their effectiveness has been traced to strong associations with water, leading to short-ranged, repulsive hydration forces, and to steric stabilization forces, which are related to the restriction of surface mobility as potential adherends approach them. With the rational molecular design of anti-bioadhesive coatings in mind, we work to decouple each of these effects by making sensitive nano-scale force measurements on organized monolayers hosting oligomers of controlled molecular weight and surface density. In this work, we employ the atomic force microscope (AFM) in two modes to obtain these two surface characteristics. Surface rheological properties, which are a measure of the propensity for steric stabilization, are obtained by driving the AFM tip across a frequency spectrum and measuring the phase and amplitude of the cantilever response as a function of probe/surface separation distance. Short-range forces are measured by operating the AFM in conventional static force modulation mode. AFM tips are functionalized to reflect pertinent biological chemistry using thiol SAM's terminated by carboxylic acids, amine groups, and the monosaccharide sialic acid.

8:40am **BI-MoM2 Probing the Local Interaction Forces with the AFM between Tertraglyme and Fluorinated Polymers and Biomolecules**, ***R. Luginbühl***, *Y.V. Pan, B.D. Ratner,* University of Washington

The interactions between biomolecules and surfaces play a major role in biological process. In biomaterial science, a key to success lays in understanding and controlling the local characteristics of the material surfaces. Engineering of recognition surfaces with well-defined chemical and physical properties is of highest interest for fabrication of biocompatible surfaces, biosensor technology, medical devices and/or molecular electronics. Polymer films, deposited in a radio frequency plasma enhanced chemical vapor deposition (RF-PECVD) process, are of increasing interest as biomaterial coatings. Plasma polymerization of tetraglyme (CH3-(O-CH2-CH2)4-O-CH3) was reported by Lopez and Ratner in 1992. The resulting thin films show resistance to protein adhesion, cell and bacteria attachment, similar to poly-(ethylene glycol) (PEG). In contrary to tetraglyme coatings, fluorine-containing films polymerized from perfluorinated monomers are very hydrophobic and exhibit a high interaction to biomolecules and cells. The interactions between biomolecules and a material surface can be probe with the atomic force microscope (AFM) at the molecular level. AFM cantilever tips were modified with a thin film of either one of the above mentioned plasma polymers and/or with biomolecules. The adhesion force was quantified by the mean of force versus displacement curves. In addition, recognition images of polymer patterned surfaces with biomolecule modified AFM tips were obtained applying a new modulation technique.

9:00am **BI-MoM3 Proteins at the Solid-Solution Interface: Significance, Behavior and Manipulation**, ***J.L. Brash***, McMaster University, Canada **INVITED**

Proteins are large, amphiphilic molecules and as such are highly surface-active: they seek out the interfacial environment. Proteins will thus tend to accumulate at any gas-liquid or solid-liquid interface, and in doing so their biological activity is often altered. Such behavior has consequences for many areas of science and technology where protein-containing fluids are present. Examples are protein separation and purification, the "biofouling" of surfaces in bioprocess equipment and biosensors, and the biocompatibility of medical devices such as vascular prostheses and contact lenses. In the case of biocompatibility, the adsorbed proteins determine subsequent cell interactions. A theme which emerges from these considerations is the need to be able to "control" or "direct" protein adsorption. In this presentation the interfacial behavior of proteins will first be discussed. Approaches to the control of protein adsorption will then be suggested. Such control has a number of aspects, including selecting a given protein from a multiprotein fluid (eg blood), controlling the conformation (and thus the function) of adsorbed proteins, and preventing adsorption altogether. Examples from the author's research in the blood compatibility area will be presented. These will include fibrinolytic (clot-dissolving) surfaces based on the preferential adsorption of plasminogen, anticoagulant surfaces based on thrombin scavenging, and protein repellent surfaces based on polyethylene oxide grafting.

9:40am **BI-MoM5 Molecular Recognition Between Genetically-Engineered Streptavidin and Surface-Bound Biotin**, ***V.H. Perez-Luna***, *K.A. Opperman, P.D. Hampton, M.J. O'Brien,* University of New Mexico*; L. Klumb, P. Stayton,* University of Washington*; G.P. Lopez,* University of New Mexico

There are fundamental differences between molecular recognition at the solid-liquid interface and in solution. In solution, ligands and receptors are randomly distributed in space, have high mobility, random orientation and, after binding, the ligand receptor pair can freely move in the solution. At the solid-liquid interface, the immobilized species is concentrated at the solid surface, has low mobility, preferential orientation for the ligand and, upon binding, the ligand-receptor pair becomes constrained to the surface region. Interactions between the immobilized biomolecules and the surface may occur or, at high densities of the immobilized receptors, attractive interactions among adjacent adsorbed ligands can give rise to cooperative effects. In this work, we study such differences with the streptavidin-biotin molecular recognition system. Binding of streptavidin to biotin-terminated self assembled monolayers (SAMs) on gold is reported. Three streptavidin mutants were used in this work: wild type, Y43A and W120A. Desorption of the bound protein molecules was obtained by incubation of the SAMs in 1 mM biotin. Desorption from disordered monolayers was incomplete, which suggests that non-specific interactions occurred either with the gold substrate or hydrophobic moieties of the thiolate after binding. Non-specific interactions did not occur on well organized monolayers and complete dissociation was achieved. Desorption of the surface bound molecules was modeled considering that the bound proteins could come off the surface either by sequential dissociation of biotin-streptavidin bonds or by simultaneous dissociation of two biotin-streptavidin bonds. The calculated dissociation constants differed by several orders of magnitude for the three mutants and they depended on the degree of coverage of surface bound biotin. The later indicating the presence of attractive interactions among adsorbed molecules at high surface coverage.

10:00am **BI-MoM6 Interfacial Influences on the Apparent Activity of Immobilized Electron Transfer Proteins**, ***D.E. Leckband***, *C. Yeung, N. Lavrik, A. Kloss,* University of Illinois, Urbana-Champaign

We determined the influence of the interfacial microenvironment on the apparent activity of immobilized proteins. In particular, we investigated the effect of the electrostatic potential of the underlying support on the interaction of soluble cytochrome b5 with immobilized cytochrome c. By varying solution pH, we controlled the magnitude of the negative charge on the supporting matrix. Because cyt b5 is also negatively charged at neutral pH, the substrate repels the soluble cyt b5 and thus opposes the cyt c/cyt b5 attraction. We show, using surface plasmon resonance, that the apparent pH-dependence of the interprotein affinity is determined largely by the pH-dependence of the substrate, and not by the intrinsic interactions between the two proteins. On the matrix used in this work, we showed that the pH-optimum for the cyt c/cyt b5 recognition shifts by 1.2 pH units relative to that of the soluble proteins. Our results demonstrate that the apparent biological activity of immobilized species must be considered within the context of the microenvironment in which they function.

10:20am **BI-MoM7 Interfacial Supra-Biomolecular Assemblies on Solid Supports**, ***W. Knoll***, The Institute of Physical and Chemical Research (RIKEN), Japan, Germany*; A. Offenhaeusser,* Max-Planck-Institut für Polymerforschung, Germany **INVITED**

This contribution summarizes some of our efforts in designing, preparing, and characterizing supramolecular interfacial assemblies integrating biomolecular functional units. Among the presented bio-interfaces are oligonucleotide matrices fabricated by self-assembly strategies based on thiol coupling to Au-substrates or on biotin-streptavidin interactions. A multispot parallel read-out of hybridization reactions between various surface-bound capture probes and complement strands from solution is presented. The concept is based on surface plasmon microscopy and image analysis computer routines. It is shown how the interfacial architecture can be optimized for maximum binding efficiency by using monomolecular layers assembled from binary thiol solutions composed of the catcher probes and diluent molecules that control the lateral separation of the individual binding sites. First results on the influence of the ionic strength,

the degree of mismatch, and the temperature are presented. In addition the extension of this concept to PNA catcher probes in discussed. The second class of surface architectures concerns tethered membranes. Various concepts for the coupling of lipid bilayers to solid supports of different materials (Au, SiOx) based on polymers or peptides are briefly discussed. Particular emphasis is put on the structural analysis of the complex multilayer assembly and on the functional characterization by electrochemical techniques. The reconstitution of membrane-integral ion translocating proteins into the supported bilayers bears great potential for biosensor formats.

11:00am **BI-MoM9 Qualitative and Quantitative Mass Spectrometric Methods for Probing Surface-Protein Binding Affinity**, ***G.R. Kinsel***, *A.K. Walker, L. Chen, K.D. Nelson, Y. Wu,* University of Texas, Arlington*; R.B. Timmons,* University of Texas, Arlington, U. S. A.

We have recently shown that Matrix Assisted Laser Desorption Ionization (MALDI) Mass Spectrometry (MS) can be used as an efficient tool for characterizing surface protein interactions. When using a standard sample preparation methodology there is a direct inverse correlation between the surface-protein binding affinity and the magnitude of the protein MALDI MS ion signal. The MALDI MS method offers numerous advantages over conventional bioanalytical methods for assaying surface-protein binding including: 1) surface binding of a broad spectrum of proteins and biomolecules can be assayed, 2) no modification (e.g. radiolabeling, fluorescent labeling) of the protein is required, 3) analysis is straightforward and can be completed in under 30 minutes 4) sensitivities are comparable to radiolabeled protein binding studies. Our present research is focused on assessment of the conditions under which the MALDI ion signal can be quantitatively correlated to the surface-protein binding affinity. Specifically we have examined the impact of changes in a variety of surface (morphology, chemical functionality, etc) and protein solution (protein choice, solution pH, etc.) characteristics on the general quantitative MALDI ion signal / binding affinity correlation. These studies reveal both the range of conditions across which the MALDI MS methodology can be applied to quantitate surface-protein binding as well as insights into the relative impact of various surface / solution parameters on the adsorption process.

11:20am **BI-MoM10 Biosensing Using Colloidal Au Arrays as Biocompatible Substrates and Au:Protein Conjugates as Signal Enhancing Agents**, ***M.D. Musick***, *L.A. Lyon, G.P. Goodrich, M.J. Natan,* Pennsylvania State University

Sensing strategies are discussed using arrays of colloidal Au as a sensor substrate and solutions of protein:Au colloid conjugates as signal amplification reagents. Colloidal Au shares similar properties with bulk Au, a common transducer substrate due to its reflectivity, conductivity, and ease of chemical modification. However, colloidal Au offers increased biocompatibility and flexibility. Particles can be assembled directly from solution onto a wide range of supports. Furthermore, particle size and spacing are easily controlled. 2-D and 3-D arrays have been fabricated from combinations of biomolecules, ligands, organic crosslinkers, and colloidal Au. Assemblies have been characterized by AFM, FE-SEM, uv-vis/NIR, electrical resistance, and electrochemical analysis. The stability and immobilization of protein:Au colloid complexes have been examined. An amplified surface plasmon resonance (SPR) sandwich assay is presented. In this assay, a protein layer immobilized on an evaporated Au film is exposed to analyte solution and incubated with a protein:Au conjugate. The result is a enhanced shift in the SPR curve as compared to conventional SPR. The use of colloidal Au amplified surface plasmon resonance should offer increased molecular weight sensitivity and lower detection limits. Imaging and arraying methods that allow for simultaneous analysis of many samples, and sensors based on changes in electrical and electrochemical signals will also be described.

11:40am **BI-MoM11 Electron Transfer of Cytochrome c on Lipid-Coated Graphite Electrode**, ***S. Boussaad***, *R. Arechabaleta, N.J. Tao,* Florida International University

The structural and electron transfer properties of Cytochrome c (Cyt c) Langmuir-Blodgett (LB) films, and Cyt c on Cardiolipin (CL) and Phosphatidylcholine (PC) monolayers have been studied on graphite electrode with tapping mode atomic force microscopy (AFM) and cyclic voltammetry. The protein in the LB film forms an ordered structure and exhibits a reversible electron transfer reaction in phosphate buffer. The analysis of the AFM images reveals a quasi-hexagonal structure with a=4.4 ± 0.2 nm, b=5.3 ± 0.2 nm and @gamma@=71 ± 3°. These dimensions are in good agreement with the X-ray data. The redox peaks of the Cyt c monolayer are about 80 mV more positive than those of the spontaneously adsorbed protein, and the electron transfer rate (20-30 s@super -1@) is smaller than 60-80 s@super -1@, the value for the adsorbed Cyt c. Furthermore, both monolayers of CL and PC are ordered on graphite, but their interactions with Cyt c are quite different. On CL monolayer, Cyt c adsorbs spontaneously and the adsorbed protein preserves the electron transfer reaction. In addition, the protein disrupts seriously the ordered structure of the lipid monolayer. However, on PC monolayer, Cyt c does not adsorb. This difference is consistent with the fact the CL plays an important role in the activity of Cyt c oxidase than PC.

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