Tuesday Morning, October 3, 2000

Biomaterial Interfaces
Room 202 - Session BI-TuM

Protein-Surface Interactions
Moderator: B.D. Ratner, University of Washington

8:20am **BI-TuM1 Quantitative Analysis of Protein Adsorption Kinetics*, V. Hlady,*** University of Utah **INVITED**

Protein adsorption from aqueous solution is determined by a "match" between two interfaces, one between the protein and the aqueous solution and the other between the adsorbent surface and the solution. A subtle interplay between polar and non-polar interactions regulates protein stability and plays a decisive role in protein interactions with the adsorbent surface. Other factors include the adsorbent's surface energetics, charge, rugosity, and the structure of water at both interfaces, i.e. their respective hydrophilicity and interfacial hydration layers. In order to characterize and predict protein adsorption, one seeks information about adsorption isotherms and kinetics, conformation of adsorbed proteins, number and character of surface-bound pr otein segments, and the physical parameters describing the adsorbed protein layer. The most powerful techniques for protein adsorption studies include optical and spectroscopic methods. These methods can provide insight into protein concentration, confor m ation and dynamics at interfaces. We have designed a spatially-resolved total internal reflection fluorescence spectroscopy method (1-D TIRF) to measure competitive adsorption kinetics of human plasma proteins. When combined with autoradiography and sur fa ce hydrophobicity gradients, 1-D TIRF experiments provide a quantitative description of protein adsorption and desorption kinetics as a function of surface hydrophobicity. As an example we will show the analysis of the adsorption kinetics from a binary so lution mixture of human serum albumin (HSA) and human low density lipoproteins (LDL) onto the model surface with a density gradient of octadecyldimethylsilyl chains on fused silica (C18-silica gradient). The adsorption and desorption rate constants are obtained by fitting the experimental results to an adsorption model that accounts for the mass transport effects and the surface density of the C18 groups.

9:00am **BI-TuM3 Investigation of the Structure and Dynamic of Proteins on Surfaces by EPR Spectroscopy: Annexin XII as an Exploratory Example*, T. Risse,*** *W.L. Hubbell,* University of California, Los Angeles*; M. Isas, H. Haigler,* University of California, Irvine

Site-directed spin labeling (SDSL) has become an important tool for the investigation of structure and dynamics in proteins. The SDSL strategy involves introduction of one or two nitroxide side chains (R1) at selected positions in the protein sequence, followed by analysis of the electron paramagnetic resonance (EPR) spectrum in terms of secondary and tertiary structure. To increase the information content of the SDSL experiment, and to examine protein structure and dynamics at interfaces, oriented arrays of spin-labeled proteins on surfaces are now under investigation. Initial experiments have employed the helical protein Annexin XII adsorbed to single lipid bilayers supported on glass or mica surfaces. For molecules oriented in 2-dimensions, the tensorial nature of the Hamilton operator gives rise to angular dependent EPR spectra which can be used to extract the orientation of the nitroxide relative to the surface. Such information is of paramount importance for determining the topography of proteins bound to surfaces. In addition, direct information on the structure and interactions of the protein at the surface is obtained from the dynamics of the side chains inferred from the spectral lineshape. Results for R1 residues at sites 213, 147, 148 and 154, 156 in oriented monolayers of Annexin XII will be discussed relative to these points.

9:20am **BI-TuM4 A Surface Plasmon Resonance Biosensor Study of Protein Interactions with Thin Apatite Films*, H.B. Lu,*** University of Washington*; B.J. Tarasevich,* Battelle Pacific Northwest National Laboratory*; C.T. Campbell, C. Giachelli, B.D. Ratner,* University of Washington

The primary objective of this research is to study protein interaction with a well-characterized apatite film using a surface plasmon resonance (SPR) sensor. Due to the remarkable osteointegration properties of apatite as an implant material, protein adsorption onto this type of material has been intensively studied. It is believed that knowledge on protein adsorption to such apatite surface will help us to understand the body's response to foreign materials and improve implant performances. However, due to the limitations of analytical tools for either material characterization or protein adsorption, few useful conclusions have been reached. In this study, a gold SPR sensor has been functionalized with a carboxylic acid-terminated self-assembled monolayer and then coated with an apatite thin film grown with the surface-induced mineralization technique. The apatite mineral films have been well characterized using surface analytical tools including TOF-SIMS, XPS, FTIR, and AFM, as well as bulk analytical technique XRD. SPR is an optical phenomenon that is very sensitive to refractive index changes perturbing the evanescent wave at metal-liquid interfaces. By coating the SPR sensor with a thin apatite film, we took advantages of continuous and label-free monitoring, and thus studied protein interactions with apatite in real time. The proteins studied include phosphorylated Rat His osteopontin (p-OPN), non-phosphorylated Rat His osteopontin (n-OPN), and bovine serum albumin (BSA). The p-OPN displayed very distinguishable adsorption/desorption behavior from that of n-OPN and BSA. The p-OPN has a higher affinity toward the apatite surface, compared to n-OPN and BSA. The p-OPN may have inhibited mineral growth upon adsorption, while BSA may have promoted slight mineral growth upon adsorption. On the other hand, these proteins behaved non-selectively when adsorbing onto control surfaces including bare gold, a COOH-terminated SAM and an NH2-terminated SAM.

9:40am **BI-TuM5 Protein Adsorption to Plasma Functionalized Surfaces Using Surface Plasmon Resonance Spectroscopy and Atomic Force Microscopy*, M.T. van Os,*** University of Twente*; A.T.A. Jenkins,* Max Planck Inst. for Polymer Res.*; M. Péter,* University of Twente*; R. Förch,* Max Planck Inst. for Polymer Res.*; R.B. Timmons,* The Univ. of Texas at Arlington*; W. Knoll,* Max Planck Inst. for Polymer Res., Germany*; G.J. Vancso,* University of Twente, The Netherlands

Plasma modification provides a powerful tool to tailor the surface properties of materials. Surface characteristics such as wettability, chemistry and morphology are known to influence protein adsorption, and the subsequent attachment and spreading of cells on biomaterials. To improve the understanding of protein-surface interactions we functionalized gold and silicon surfaces with amino or ether groups, using radio frequency plasma polymerization of ethylenediamine, allylamine, cycloheptylamine and di(ethyleneglycol)vinylether (EO2V). The functional group density at the surface was controlled by using different monomers or by variation of the input power during the plasma deposition. The adsorption of the proteins fibrinogen, bovine serum albumin and immunoglobulin G to these surfaces was measured in situ with surface plasmon resonace spectroscopy. The tenacity of the protein adsorption on the different substrates was also measured, after removing elutable protein with 1% sodium dodecyl sulfate (SDS) solution. After drying, the protein layers were studied by tapping mode atomic force microscopy (TM-AFM). The results obtained show that both the protein adsorption to and the retention on the surfaces are affected greatly by the surface functionalities. All the amine functionalized surfaces showed a high affinity toward the proteins, and thin dense layers of adsorbed protein remained on these surfaces, even after rinsing with SDS solution. A large contrast in protein affinity was observed between the EO2V films polymerized at different power input conditions. A dramatic reduction in both initial adsorption and retention of all proteins was observed on these films with decreasing power. The low degree of cross-linking, as well as the high retention of ether content during the polymerization of EO2V under low power input conditions is thought to result in the production of biologically non-fouling surfaces.

10:00am **BI-TuM6 How to Make and Analyze Cross-linked Monolayers of Mytilus Edulis Foot Proteins (Mefp)*, H. Elwing,*** *K. Mjorn,* Lab of Interface Biophysics, Sweden*; K. Uvdal, M. Fahlman,* Linkoping University, Sweden*; J Lausmaa,* National Testing and Res. Institute, Sweden*; F. Hook,* Lab of Interface Biophysics, Sweden

The Mefp proteins are potential candidates as "tissue glues" in biomaterial applications. Several of the Mepf proteins contain high amounts of DOPA (dihydroxyphenylalanin). On oxidation to o-quinone the DOPA molecules become highly reactive and forms a base for cross-linking of the proteins in the byssus threads as well as binding to solid surfaces. We have developed an experimental model consisting of polar siliconoxide surfaces and apolar alkanethiol surfaces. On this surfaces we follow adsorption of purified Mefp-1 with the use of optical methods such as surface plasmon resonance (SPR) and high precision ellipsometry. Periodate induced crosslinking of the molecular layers is then followed by Quarts crystal microbalance (QCM-D) and ellipsometry. At apolar surfaces we found a reduction of layer thickness from about 20 nm to about 4 nm as well as a significant reduction of the viscoelastic properties of the protein layers as measured by QCM-D. On the other hand, adsorption of Mefp-1 on polar surfaces res ulted in a protein layer that was thin and dense from the beginning and cross-linking resulted only in significant small change of layer thickness and

viscoelasticity of the mefp-1 layer. It was obvious that adsorption of Mefp-1 to polar surfaces significantly reduced the possibility of cross-linking most probably due to binding engagement of the DOPA side chains to the silicon oxide surface. We also made an analysis with photoelectron spectroscopy (XPS). High-resolution spectra at normal and glancing take off angles were obtained with a particular emphasis placed on the C 1s core level. Four distinct peaks were visible in the non-deconvoluted spectrum. Carbon-nitrogen and carbon-oxygen bonds were studied as a function of depth, crosslinking and the polarity of the substrate. Significant differences were found and is now subjected to a more detailed analysis together with data from time of flight secondary ion spectroscopy (TOF-SIMS).

10:20am **BI-TuM7 Assessment of Fibronectin Conformation Adsorbed to Polytetrafluoroethylene Surfaces from Serum Protein Mixtures and Correlation to Support of Cell Attachment in Culture**, ***D.W. Grainger***, Colorado State University; *G. Pavon-Djavid, V. Migonney, M. Josefowicz,* Universite Paris, France

Fluoropolymer surfaces in biotechnology applications are notorious for tightly adsorbing proteins that do not support cell attachment. Reasons for this remain confusing but surround both the population and conformation of proteins adsorbed competitively from physiological milieu that do not interact with cell adhesion receptors. In this study, polytetrafluoroethylene (PTFE) surfaces were exposed to buffered aqueous solutions containing radio labeled (@super 125@I) human fibronectin (Fn), fibronectin:serum albumin (BSA) binary mixtures of various ratios, or whole human plasma dilutions (one hour). Total adsorbed fibronectin and albumin following rinsing were quantified on PTFE. @super 125@I-labeled monoclonal antibodies against either the fibronectin cell adhesion (containing the RGDS integrin recognition motif) or the fibronectin amino terminal domains were used to probe accessibility of each of these fibronectin regions post-adsorption. Human umbilical vein endothelial cells (HUVECs) were then cultured on PTFE surfaces pre-exposed to each of these protein adsorption conditions and compared to identical conditions on tissue culture polystyrene (TCPS) controls. Fibronectin adsorption to PTFE is dependent upon the concentration of albumin co-adsorbing from solution: albumin out-competes fibronectin for PTFE surface sites even at elevated nonphysiological Fn:HSA ratios. Antibodies against Fn do not readily recognize Fn adsorbed on PTFE as the HSA co-adsorption concentration in either binary mixtures or in plasma increases, indicating albumin masking of adsorbed Fn. At higher Fn:HSA ratios, albumin co-adsorption actually improves anti-Fn antibody recognition of adsorbed Fn. HUVEC attachment efficiency to PTFE after protein adsorption correlates with amounts of Fn adsorbed and levels of anti-Fn antibody recognition of Fn on PTFE, linking cell attachment to integrin recognition of adsorbed Fn density and Fn adsorbed conformation on PTFE surfaces.

10:40am **BI-TuM8 Protein Adsorption on Self-assembled Polyelectrolyte Multilayer Films**, *G.D. Ladam,* Institut Charles Sadron, France; *F.J.G. Cuisinier,* Federation de Recherche "Odontologie", France; *G. Decher,* Institut Charles Sadron, France; *J. Voegel,* Federation de Recherche "Odontologie", France; ***P. Schaaf***, Institut Charles Sadron, France

Alternating polyelectrolyte films were constructed by the sequential adsorption of poly(allylamine hydrochloride) (PAH) and polystyrenesulfonate (PSS) onto a silica surface. The film build-up and the further adsorption of proteins (human serum albumin (HSA), ribonuclease A, lysozyme, alpha-lactalbumine, myoglobine) were followed in situ versus time by means of scanning angle reflectometry. We investigated first the influence of the isoelectric point of the proteins on their adsorption onto positive (PAH ending) and negative (PSS ending) multilayers. At a protein concentration of 0.25mg/ml at pH 7.4 and in the presence of Tris HCl 10-2M, 0.15M NaCl buffer, all proteins adsorbed on both positive and negative polyelectrolyte films with thicknesses varying from the monolayer or less, up to thicknesses equivalent to at least 4 protein layers. Thick protein layers were observed when proteins and films were oppositely charged. The adsorption of HAS onto both films was investigated as a function of the protein concentration and the NaCl concentration of the adsorbing solution. It was found that on PSS ending multilayers exhibiting a similar charge as albumin, the proteins still adsorb but only a monolayer can be reached. On the other hand, on PAH ending multilayers thick protein films are observed. The adsorbed amount depends also critically on the NaCl concentration of the adsorbing solution. Desorption experiments were also performed and depending on the salt concentration of the rinsing solution one can observe no desorption or partial desorption (up to 50% of the adsorbed amount). A microsocopic model will be discussed trying to explain these experimental findings.

11:00am **BI-TuM9 Desorption/Ionization Mass Spectrometry on Porous Silicon Surfaces**, ***Z. Shen***, *J.E. Crowell,* University of California, San Diego; *G. Siuzdak,* The Scripps Research Institute

A new desorption/ionization strategy for biomolecular mass spectrometry has been developed based on pulsed laser desorption/ionization from a porous silicon surface. Desorption/ionization on silicon (DIOS) uses porous silicon to trap analytes deposited on the surface and laser radiation to vaporize and ionize these molecules. DIOS is demonstrated for a wide range of small molecules as well as biomolecules at the femtomole and attomole level with little or no fragmentation, in contrast to what is typically observed with other direct desorption/ionization approaches. Porous silicon surfaces were prepared using electrochemical etching. While DIOS has been universally applicable for a range of mass analyses, its success is highly dependent upon the preparation of the sample and the nature of the porous silicon surface. Different etching parameters, including silicon wafer crystal orientation, dopant type, dopant level, light intensity, current density, etching solution, and etching time were studied to optimize DIOS-MS performance. Scanning Electron Microscopy (SEM) was used to examine the pore structure and correlate it with DIOS-MS performance. We will also demonstrate the application of DIOS-MS to small molecule analysis and quantitation, protein identification, on-chip reaction monitoring, on-chip separation and post-source decay structure analysis. DIOS offers many unique advantages including good sensitivity, low background ion interference, and high salt tolerance. Desorption/ionization on porous silicon (DIOS) permits analysis of a wide range of molecules with very good sensitivity and a demonstrated potential for automation, as well as compatibility with microfluidics and microchip technology on silicon.

11:20am **BI-TuM10 Determination of Surface-Protein Equilibrium Binding Constants by MALDI Mass Spectrometry**, ***G.R. Kinsel***, *J. Zhang, R.B. Timmons, H. Qiu,* University of Texas at Arlington

We have recently demonstrated that matrix assisted laser desorption / ionization (MALDI) mass spectrometry offers a new approach for the characterization of surface-protein interactions. Our work demonstrates that strongly surface-retained proteins are poorly incorporated into the MALDI matrix crystals, leading to inefficient ionization of these species. In effect, the surface-deposited protein MALDI ion signal approaches zero as the quantity of deposited protein approaches the quantity strongly retained by the surface. Furthermore, as expected, the protein MALDI ion signals exhibit Langmuir type behavior as the surface concentration of the protein is reduced, i.e. the protein MALDI ion signal versus surface concentration response becomes asymptotic at low protein surface concentrations. Analysis of the protein MALDI ion signal versus surface concentration data allows the equilibrium surface-protein binding constant to be established. In our current work this approach to the determination of surface-protein binding constants has been applied to a number of smaller peptides and proteins deposited on a variety of polymeric biomaterials. Expected trends are observed, particularly with regard to the influence of electrostatic interactions between acidic or basic surfaces and basic or acidic proteins in solution. In addition, we have used the MALDI approach to examine the elutability of surface-bound proteins as a function of solvent choice. Our studies indicate that protein solubilization as a function of solvent choice is strongly influenced by the chemistry of the surface-protein interaction.

11:40am **BI-TuM11 The Molecular Orientation Distribution of an Electrochemically Active Protein Monolayer Adsorbed to Indium-Tin Oxide**, *S. Saavedra,* ***R.T. Robertson***, *S.B. Mendes, N.R. Armstrong,* University of Arizona

The relationship between molecular orientation and heterogeneous electron transfer behavior in immobilized films of redox-active proteins is being investigated using absorbance and fluorescence techniques that combine the information content of spectroele ctrochemistry with the sensitivity of the single-mode, planar waveguide geometry. Spectroelectrochemistry of surface confined, redox-active films can be performed with a pathlength enhancement of approximately 4,000 relative to a transmission geometry. T he use of this approach to determine the tilt angle distribution of the porphyrin molecular planes in a submonolayer of electrochemically active cytochrome c adsorbed to an indium-tin oxide electrode will be described. Developing a better understanding of the relationship between protein film structure and redox activity may aid efforts to rationally design protein-based molecular devices in which control of vectorial electron transfer is a prerequisite for efficient operation.

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