Tuesday Morning, October 16, 2007

Biomaterial Interfaces

Room: 609 - Session BI-TuM

Proteins at Interfaces

Moderator: D.G. Castner, University of Washington

8:00am **BI-TuM1 Inter-Domain Structural Flexibility and Biological Activity of Pro-Matrix Metalloproteinase-9 Revealed by Single-Molecule AFM Imaging**, *G. Rosenblum*, ***S.R. Cohen***, *J. Frenkel*, Weizmann Institute of Science, Israel, *N. Slack*, Veeco Metrology Division, Santa Barbara, *I. Sagi*, Weizmann Institute of Science, Israel

The multi-domain enzyme pro-matrix metaloproteinase-9 (pro-MMP-9) is recognized as playing a key role in tumor biology, autoimmune diseases, and vascular pathology. This enzyme cannot be crystallized and hence the only structural information available is of the two isolated terminal domains. Until now, structure of the vital linker domain that connects these terminal domains was unknown. A lack of reliable means to bind the protein to the surface has plagued previous structural characterization by high-resolution AFM imaging. In order to obtain high-quality AFM images of the small protein, novel amine-modified surfaces were employed to immobilize the protein during the extensive rinsing required for removing features due to buffer salts. AFM images presented in this work provide the first definitive confirmation of the multi-domain structure, wherein two terminal domains are connected by a linker segment. Parallel analysis of a mutant lacking the linker showed a less extant shape. Statistical analysis of the AFM images revealed differences in both heights and lengths between the native and mutant proteins, and provided evidence that the linker imparts significant conformational freedom to the molecule, which is likely important in its biological functioning. Biological functioning was further probed, by examining interaction of the enzyme with collagen. Molecular modeling based on the SAXS data provides complementary supporting data.

8:20am **BI-TuM2 Sum Frequency Generation Vibrational Spectroscopic Studies in the C-H, O-H, N-H, and Amide I Regions of Model Peptides at Solid-Liquid Interfaces**, ***R.L. York***, *G. Holinga*, *W.K. Browne*, *C. Hahn*, Univ. of California, Berkeley and LBNL, *D.R. Guyer*, LaserVision, Inc., *K.R. McCrea*, *R.S. Ward*, The Polymer Technology Group, *G.A. Somorjai*, Univ. of California, Berkeley and LBNL

We have developed a library of small, model peptides and have examined their interfacial structure at model hydrophobic and hydrophilic surfaces using surface-specific sum frequency generation vibrational spectroscopy (SFG). A fourteen amino acid peptide containing hydrophobic leucine (L) and hydrophilic lysine (K) residues was synthesized and characterized. This amphiphilic α-helical peptide has sequence Ac-LKKLLKLLKKLLKL-NH2 (LK14). SFG spectra in the C-H, O-H, and N-H region reveal that at a hydrophobic deuterated polystyrene surface, methyl groups from the hydrophobic leucine residues are ordered at the interface, while the hydrophilic lysine residues adopt a random orientation (presumably due to lack of interaction with the surface). When adsorbed onto a hydrophilic silica surface, the SFG spectra reveal a completely different molecular orientation: the methyl groups now appear to have a random orientation, and the N-H groups of the lysine side chains and/or peptide backbone are now ordered. A study of the influence of the ionic strength of the solution on the structure of LK14 revealed the following results: the LK14 peptide was α-helical in solution at high ionic strength but random coil at low ionic strength. Furthermore, leucine side chain ordering on hydrophobic surfaces was not perturbed by ionic strength changes, but N-H ordering on hydrophilic surfaces had a strong dependence on ionic strength. More recently, we have developed a new optical parametric amplifier (OPA) utilizing lithium thioindate (LIS) to study the Amide I mode of the peptide backbone. LIS provides high IR energy (~175 μJ at 1500 cm-1, ~375 μJ at 2000 cm-1), a high damage threshold, and good beam quality. The high energy output of LIS allows for the study of interfacial peptide structure without having to use a total internal reflection geometry. Using this new OPA, we have seen evidence for α-helical peptide structure at both hydrophobic and hydrophilic surfaces (at high ionic strengths). Additionally, there appears to be evidence for α-helical structure of the LK14 peptide at hydrophobic surfaces in low ionic strength solutions. We are currently examining lysine homopeptides, collagen-like peptides, new experimental geometries, more biologically relevant surfaces (such as HEMA and polymers with Surface Modifying Endgroups) and molecular dynamics simulations of peptides at interfaces to aid interpretation of experimental data.

8:40am **BI-TuM3 Thin Hydrogel Layers on Biomedical Polymers - Biological Responses and Effects on Protein Adsorption Studied by Mass Spectrometry**, ***D.L. Elbert***, Washington University **INVITED**

Medical devices used in contact with blood often contain features that are on the order of microns, (e.g. the struts on stents). Application of thin polymeric coatings on stents is feasible for the release of antiproliferative agents. However, the use of hydrogel coatings in this application is limited by a number of factors. Thin hydrogel coatings may be difficult to apply on complex geometries. The large volume of water in the hydrogel also limits the amount of drug that can be delivered from the coating. To address the first issue, we previously synthesized a copolymer of polylysine and polyethylene glycol (PLL-g-PEG) that self-assembles on negatively charged surfaces. We demonstrated that very thin yet stable layers of water-soluble polymer reduce biological responses, both in vitro and in vivo. We followed these experiments with investigations into the uses of layer-by-layer strategies, however, practical utility of these films is limited by time-prohibitive methods of fabrication, and the formed films may be too thin for drug delivery. We have addressed these shortcomings in two ways. Rather than delivering drugs directly, we are incorporating an enzyme into hydrogels. The enzyme produces a biologically active molecule (sphingosine 1-phosphate) from a precursor already present in blood (sphingosine). This molecule causes endothelial cell chemotaxis and inhibits smooth muscle cell migration. Additionally, we are producing multilayer films from nanogels that are formed by crosslinking PEG-vinylsulfone with albumin (average particle sizes 40 - 80 nm). Even a single layer of the nanogels covalently-reacted with RFGD-modified PET greatly reduces cell adhesion. Finally, in characterizing protein adsorption on thin hydrogel films, it is important to know not only the amount of adsorbed protein but also the conformations adopted by the adsorbed proteins. To study this, we have developed a proteomics-based strategy to detect differences in the exposure of lysine residues following adsorption. Our studies demonstrated an increased accessibility of lysine residues in fibrinogen adsorbed from low concentration solutions, which correlated well with the increase in the spread area of fibrinogen as measured at the same solution concentrations by OWLS. Overall, tremendous challenges and opportunities exist for producing thin surface coatings that resist non-specific biological adhesion and deliver drugs to control the biological response.

9:20am **BI-TuM5 Development of a Molecular Modeling Program Specifically Designed for the Simulation of Protein Adsorption to Biomaterials Surfaces**, ***R.A. Latour***, *P. Biswas*, Clemson University, *B.R. Brooks*, Laboratory of Computational Biology - NIH, *S.J. Stuart*, Clemson University

Although it is well understood that cellular responses to biomaterial surfaces and substrates for tissue engineering and regenerative medicine are primarily governed by the bioactive state of adsorbed proteins, very little is known regarding the molecular-level events involved in these processes. Without this understanding, surface design can only be approached by trial and error. Molecular simulation provides a means to overcome this problem by providing an approach to directly investigate protein-surface interactions at the molecular level. Molecular simulation methods have already been successfully developed and widely applied for the study of protein folding and for drug design. However, these methods are not suitable for protein-surface interactions studies as it involves solid-liquid multiphase interactions which must be addressed specifically. Also, force-fields specifically designed for solid or liquid phase are not easily transferable. We are therefore working to develop capabilities within the CHARMM molecular simulation program to specifically adapt it for the simulation of protein adsorption processes to biomaterials surfaces. In particular, capabilities are being developed to control the solid phase, the solution phase, and the interactions between them with three separate force fields, thus enabling the molecular behavior of each phase of the system to be accurately represented. While force field parameters for proteins in solution and various solid materials have previously been developed and validated, parameters for the interactions between proteins in solution and functional groups presented by a solid surface have not. As an integral part of this program, we are therefore also generating experimental data on peptide-surface interactions for a wide range of amino acid residues and polymer-like functional groups for the design and validation of an interfacial force field for use in the developed program. In this presentation, we will describe the modifications in the CHARMM code and results exhibiting the usefulness of this hybrid force field approach for the simulation of peptide and protein interactions with a solid surface. Once fully developed, this

approach holds promise to provide the biomaterials field with an exciting new tool to proactively design biomaterials surfaces to direct cellular response by controlling the bioactive state of adsorbed proteins with broad application in biomedical engineering and bionanotechnology.

9:40am **BI-TuM6 Switching the Force between a Hydrophobic Probe and Self-Assembled Monolayers on Gold by Changing the Ionic Strength**, ***N. Bonnet***, *D. O'Hagan, G. Hähner*, University of St Andrews, Scotland, UK

Alkanethiol based self-assembled monolayers (SAMs) have seen an ever increasing interest since they were first introduced more than two decades ago. Part of their attraction is due to the ease of their preparation in combination with the great flexibility they offer to create concentrated planes of functionality by modification of the surfactant molecules. One prominent example are oligo(ethylene glycol) (OEG)-terminated alkanethiol SAMs. They have been shown to resist the non-specific adsorption of some proteins and hence are of significant interest in the life sciences and related areas. Inspired by their protein repelling properties several studies were carried out to determine the underlying mechanisms and the interactions involved. The forces measured with a hydrophobic probe (attraction or repulsion) on these films were found to correlate with the protein adsorption properties (adsorption or resistance to adsorption). The forces indicated the existence of an electrical double layer, suggesting that an electrostatic component is involved. This was confirmed by theoretical calculations. We report on surfactant films based on OEG modified alkanethiol SAMs that can switch between an attractive and a repulsive force with hydrophobic probes depending on the ionic conditions of the environment. The ionic strength is an external parameter that can be easily controlled. The films are interesting for the reversible immobilization of hydrophobic (nano)particles and in colloidal chemistry. Similar surfactants might become important in bio-related fields and in connection with biosensors since they have the potential to adsorb/immobilize proteins reversibly if the switch can be tailored to occur in an ionic range that is compatible with proteins.

10:40am **BI-TuM9 When Good Cholesterol Goes Bad: Proteins at the Water:Lipid Interface**, ***J.W. Heinecke***, University of Washington **INVITED**

Apolipoproteins are amphipathic alpha helical proteins that play a key role in lipid transport in biological systems. HDL - the good form of blood cholesterol that protects against heart disease - is a complex of apolipoprotein A-I (apoA-I), free cholesterol, phospholipids and neutral lipids. Posttranslational oxidative modifications of apoA-I have been proposed to play a pathogenic role in atherosclerosis. Quantifying oxidized amino acids in atherosclerotic tissue proteins by isotope dilution gas chromatography mass spectrometry (GC-MS) has been used to assess oxidative stress in vivo and to investigate the biochemical pathways that contribute to inflammatory disease. These studies have identified myeloperoxidase, a heme protein secreted by activated phagocytes, as one important pathway for oxidizing HDL in the human artery wall. Electrospray ionization tandem mass spectrometry (ESI-MS/MS) coupled with liquid chromatography is rapidly emerging as a powerful technique for pinpointing sites of amino acid oxidation within apolipoproteins. Oxidants generated by myeloperoxidase lead to the site specific oxidation of apoA-I, and these posttranslational modifications are associated with major impacts on the biological function of the proteins. We have recently used 2-dimensional liquid chromatography ESI-MS/MS to test the hypothesis that proteins implicated in inflammation might be enriched in the HDL of subjects with coronary artery disease (CAD). Our observations suggest that HDL carries a unique cargo of proteins in CAD subjects and that certain of these proteins might make previously unsuspected contributions to the anti-inflammatory properties of HDL.

11:20am **BI-TuM11 In situ Quartz Crystal Microbalance Studies of Multi-Layer Glucagon Fibrillation at the Solid-Liquid Interface**, ***M.B. Hovgaard***, *M. Dong*, University of Aarhus, Denmark, *D.E. Otzen*, University of Aalborg, Denmark, *F. Besenbacher*, University of Aarhus, Denmark

The ability of certain polypeptides to form amyloid aggregates and their subsequent deposition at plague sites has been associated with multiple protein folding disorders.[1] As in vivo conditions associated with amyloid fibrillation are often dictated by surface and lipid membrane phenomena, the study of amyloid fibrillation at the solid-liquid interface represents an important and physiologically relevant approach to the study of amyloid fibrillogenesis. We have used Quartz Crystal Microbalance with Dissipation (QCM-D) to monitor the changes in layer surface mass density and viscoelastic properties accompanying multi-layer amyloid deposition in situ for the first time. By means of Atomic Force Microscope (AFM) imaging, an unequivocal correlation is established between the interfacial nucleation and growth of glucagon fibrils[2] and the QCM-D response. The combination of the two techniques allows us to study the temporal evolution of the interfacial fibrillation process. We have modelled the QCM-D data using an extension to the Kelvin-Voigt viscoelastic model. Three phases were observed in the fibrillation process: I) A rigid multilayer of glucagon monomers forms and slowly rearranges; II) This multilayer subsequently evolves into a dramatically more viscoelastic layer, containing a polymorphic network of micrometer long fibrils growing from multiple nucleation sites; III) The fibrillar formation effectively stops, due to the depletion of bulk phase monomers, although the process can be continued without a lag phase by subsequent addition of fresh monomers. The robustness of the QCM-D technique, consolidated by complementary AFM studies, should make it possible to combine different components thought to be involved in the plaque formation process and thus build up realistic models of amyloid plaque formation in vitro.

[1]Frokjaer, S. and D.E. Otzen, Protein drug stability: A formulation challange, Nat Rev Drug Discov. 4, 298-306 (2005).

[2]M. Dong, M. B. Hovgaard, S. Xu, D. E Otzen, and F. Besenbacher, AFM Study of Glucagon Fibrillation Via Oligomeric Structures Resulting in Interwoven Fibrils. Nanotech. 17, 4003-4009 (2006).

11:40am **BI-TuM12 Modulating the Orientation and Conformation of Bone Osteopontin and Bone Sialoprotein for Osteoblast Adhesion**, ***M.T. Bernards***, *S. Jiang*, University of Washington

Bone tissue is primarily composed of hydroxyapatite (HAP), which accounts for 70-90% of the mass of bone. The remaining 10-30% is composed of protein, of which ~90% has been shown to be collagenous. During natural bone formation cells first lay down a collagenous matrix composed of primarily type 1 collagen. After this collagen network is formed, proteins bind to the matrix and then the matrix is mineralized to form bone. While a number of proteins have been located within bone, only osteopontin (OPN) and bone sialoprotein (BSP) have been localized in the matrix ahead of the mineralization front. Additionally, both of these proteins have been found to be enriched at bone-implant interfaces. These two proteins are hypothesized to play an important role in cellular adhesion at these interfaces. This work details efforts to understand the roles of OPN and BSP in cellular binding to engineered systems mimicking the collagen and mineralized interfaces of bone. In the first part of this work, we compared the cell binding abilities of OPN and BSP when specifically bound to collagen. Both of these proteins have been shown to have a specific binding interaction with collagen and this was confirmed through the development of radiolabeled adsorption isotherms for each of the proteins. These isotherms were used to determine adsorption parameters that resulted in identical amounts of adsorbed protein, to directly compare the cell binding properties of these two proteins when specifically bound to collagen. The results indicate that OPN promotes a greater amount of cell binding to a collagen interface than BSP. The second part of this work compared the cell binding abilities of OPN and BSP when they are specifically bound to HAP, mimicking mineralized bone interfaces. HAP was formed from a simulated body fluid and characterized by scanning electron microscopy, energy dispersive x-ray spectroscopy, and electron spectroscopy for chemical analysis. Both OPN and BSP have been shown to have a specific HAP binding domain and this was confirmed through the development of radiolabeled adsorption isotherms. The cellular adhesion to HAP was then compared with identical amounts of adsorbed proteins. The results of this work indicate that BSP has a more favorable orientation/conformation for cellular adhesion as compared to OPN. However, this influence on cell binding is eliminated when the surface roughness of the underlying HAP substrate becomes too great.

12:00pm **BI-TuM13 Characterization of ECM Protein Production in Spatially Cultured Hippocampal Neurons on Micro-Patterned Surfaces in Serum-Free Conditions**, ***M. Ramalingam***, *S. Kootala, N. Bhargava, M. Stancescu, M. Hirsh-Kuchma, M. Klimov, P. Molnar, J.J. Hickman*, University of Central Florida

Spatial positioning of neurons on patterned surfaces and characterization of their functional synaptic connectivity and specific extra-cellular matrix (ECM) protein productivity is of great importance for the fabrication of neuron-based biosensors and in developmental cell biology. We have determined that a combination of traditional biological analysis techniques, such as SDS-PAGE and PT-PCR, and surface analytical techniques, such as X-ray Photoelectron Spectroscopy (XPS) and SIMS, is a good approach for ECM analysis. Here, we report on ECM deposition on patterns of hippocampal neurons on surfaces composed of self-assembled monolayers (SAMs) of two different organic compounds, trimethoxysilylpropl-diethylenetriamine (DETA) and tridecafluoro-1,1,2,2,-tetrahydroctyl-1-tricholorsilane (13F) as well as unpatterned controls, in a serum-free culture condition. The patterns were characterized by XPS, contact angle goniometry, electroless metallization and surface profilometry to confirm their surface composition, wettability and topography. Immunostaining of cultured neurons for synapsin I and microtubule-associated proteins (MAP-2) confirmed the pre- and post-synaptic formation. The electrophysiological study of neurons cultured for 14 days further confirmed the functional

synaptic connectivity. The deposition and composition of ECM proteins were determined by immunocytochemistry, confocal laser spectroscopy and reverse transcriptase –polymerase chain reaction (RT-PCR), and it was found that the neurons produce laminin, collagen, fibronectin and vitronectin at differing amounts depending on the conditions. We have quantified the amounts of these proteins using Western Blot and SIMS spectroscopy. The overall results indicate that the neurons cultured on patterns secrete ECM proteins in a differential fashion and these data will have significant implications in engineering functional neuronal systems and hybrid devices.

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