Wednesday Afternoon, October 20, 2010

Biomaterial Interfaces
Room: Taos - Session BI-WeA

Proteins & Peptides on Surfaces
Moderator: H.E. Canavan, University of New Mexico

2:00pm **BI-WeA1 Interaction of Amphiphilic Antimicrobial Peptides with Phospholipid Membranes, Bacteria, and Cells**, ***M. Malmsten***, Uppsala University, Sweden **INVITED**

Due to increasing resistance development, antimicrobial peptides (AMPs) are receiving increasing attention since these may provide rapid and broad-spectrum response to a host of pathogens. In addition, some of these peptides provide also strongly antiinflammatory responses, and are therefore promising in therapies of both acute and chronic inflammation. Critical for their antimicrobial action is the interaction between AMPs and bacteria membranes, where significant current efforts are directed to identifying peptides being potent antimicrobials, yet simultaneously displaying low toxicity. In inflammation, additional aspects are of importance, including interaction with lipopolysaccharide and other bacterial components. In our efforts to adress these and other challenges in the development of such peptides to practical therapeutics, our research addresses various aspects of interaction of AMPs with lipid membranes, bacteria, and cells. Focusing on endogenous peptides generated during normal microbial infections, we combine basic biophysical investigations on various aspects of AMP-membrane interactions with modern biotechnological tools for peptide design, and with biological experiments including bacteria, cells, and various animal models. Some recent examples of the work done in these contexts will be provided, aiming at synthesizing biophysical and biological aspects of these peptides.

2:40pm **BI-WeA3 Curcumin Offers Neuroprotection by Inhibiting amyloid-β Insertion into Membranes**, ***A. Thapa***, *B. Gilver, E.Y. Chi*, University of New Mexico

Alzheimer's disease (AD) is a major cause of dementia in elderly people, affecting 5 million people in USA alone. AD is caused by the abnormal accumulation of aggregated amyloid beta peptides (39 to 43 amino acid residues) in the brain. Amyloid beta peptides are proteolytic products of the amyloid precursor protein of unknown function. Unfortunately, there are no cures available for this disease. However, there are several mechanisms purposed to cause and cure AD. The lipid membrane has been shown to mediate the fibrillogenesis and toxicity of Alzheimer's disease amyloid beta (Aβ) peptide. Several reports have linked the insertion of Aβ peptide into membranes as a possible mechanism of neurotoxicity. We hypothesized that small molecules capable of preventing the insertion of Aβ into membranes may ameroliate Aβ toxicity. Therefore, we investigated the effect of curcumin, a naturally occurring anti-inflammatory and antioxidant compound that suppresses oxidative damage, inflammation, cognitive deficits, and amyloid accumulation, on Aβ40 induced toxicity and in Aβ40 insertion into 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG) monolayer using surface pressure insertion isotherms and fluorescence microscopic techniques. We found that curcumin attenuates Aβ40 induced neuronal toxicity by inhibiting the insertion of Aβ into membranes possibly by interacting with membranes. Our data also demonstrated that neuroprotective action of curcumin in Aβ induced toxicity does not exclusively come through oligomerization inhibition, indicating that c urcumin-membrane interaction but not curcumin-Aβ is associated in curcumin mediated neuroprotection. Altogether, our study suggests that curcumin-like small molecules inhibitors of Aβ insertions into membranes could be potential target to cure AD.

3:00pm **BI-WeA4 Nonspecific Protein Adsorption Requires Large Adhesive Domains on the Surface**, ***L. Shen***, *X.-Y. Zhu*, University of Texas, Austin

We study the dynamics of protein adsorption using nm – mm scale patterns involving hydrophobic domains in hydrophilic matrices. We report the discovery of a critical requirement on the sizes of adhesive pads for protein adsorption: the area of each adhesive pad must be more than two orders of magnitude larger than the footprint of a protein molecule before irreversible adsorption occurs . We attribute this to the minimal surface area sampled by a mobile protein molecule in a precursor state before irreversible adsorption occurs.

4:00pm **BI-WeA7 Study of Adsorption and Orientation of $FnIII_{7-10}$ Fibronectin Fragment on Self-Assembled Monolayers using Time of Flight Secondary Ion Mass Spectrometry**, ***L. Árnadóttir***, *J. Brison, L.J. Gamble*, University of Washington

Protein adsorption and orientation plays a critical role in many biomedical applications. Fibronectin (FN) is an extracellular matrix protein that is involved in many cell processes such as adhesion, migration and growth. The orientation and conformation of FN adsorbed onto surfaces can therefore play a critical role on cell-surface interactions. In this study, the adsorbed orientation and conformation of the 7-10 fragment of FNIII was studied on three different model surfaces (self-assembled monolayers (SAMs) of C_{11} alkanethiols on Au with $-CH_3$, $-NH_2$, and -COOH functional groups). X-ray photoelectron spectroscopy (XPS) was used to quantify the amount of protein adsorbed on the different surfaces and time-of-flight secondary ion mass spectrometry (ToF-SIMS) was used to characterize their orientation and conformation. A trehalose coating was also used to inhibit the conformation changes due to the dehydration of the sample. With the help of principal component analysis (PCA), the peaks which are responsible for the variance observed between the spectra relative to the protein adsorbed on the different surfaces could be identified. Because the surface sensitivity of the ToF-SIMS technique is lower than average protein size, these changes in the spectra reflect differences in the conformation and the orientation of the FN fragment. Comparison of trehalose protected and unprotected samples show a significant difference in the ratio between hydrophilic and hydrophobic amino acids. The results suggest that the more hydrophilic amino acids stay on the outside of the trehalose protected protein while the more hydrophobic ones get exposed to the protein air interface upon drying. Comparison of the trehalose protected fragment on $-CH_3$ and -COOH terminated SAMs show more intense signals of Arg and Asp on the -COOH surface and more intense Val, Pro and Leu signals on the $-CH_3$ SAMs. The detection of these different amino acids for the protein on the different SAMs suggests that the fragment might partly denature upon adsorption to the hydrophobic surface.

4:20pm **BI-WeA8 Temperature Controlled Dehydration of Protein Films: Time-of-flight Secondary Ion Mass Spectrometry Study of Conformational Mobility of Proteins in Vacuum**, ***H.P. Bui***, *T.P. Beebe, Jr.*, University of Delaware

Once a biomaterial is implanted, a film of protein will adsorb onto the surface and it is this protein film that will dictate how the biomaterial will interact with the surrounding cells and tissue. Two strategies to increase the success rate of biomaterials are to passivate the surface so that it is resistant to protein adsorption, or to activate the surface to obtain a desired cell response. One method of activation is the grafting of one or more proteins onto the surface that direct specific cellular interactions. However a thorough understanding of the protein film's composition, conformation and orientation is needed in the development of these advanced biomaterials. Time-of-flight secondary ion mass spectrometry's (TOF-SIMS) high surface specificity, analytical sensitivity and ability to provide long-range molecular information can be used to probe protein composition, conformation and orientation. In this study, we demonstrate that the use of temperature-programmed dehydration and principal component analysis can be used as a method of determining the conformation and orientation of protein films. We have found that TOF-SIMS is sensitive to the dehydration of the protein film and the accompanying conformational changes

4:40pm **BI-WeA9 Structure and Function of von Willebrand Factor on Glass, Polystyrene, and Tissue Culture Polystyrene**, ***E. Hillenmeyer***, *R.A. Penkala, W. Thomas, D.G. Castner*, University of Washington

von Willebrand Factor (vWF) is a blood-soluble clotting protein responsible for binding platelets through the glycoprotein 1b (GP1b) receptor on the platelet surface. vWF can become activated and bind platelets when bound to exposed collagen in blood vessels or when vWF experiences increased shear.

vWF can also bind platelets when adsorbed to synthetic surfaces and participate in clot formation, which is not desirable for blood-contacting biomaterials. There is evidence that surface properties can influence vWF adsorption. Previous studies showed differences in protein topography (1) and conformation (2) when vWF was adsorbed on mica (1), octadecyltrichlorosilane modified glass (1,2), and collagen VI (2). However, studies were not performed to relate adsorption differences to vWF function.

To more fully characterize the adsorption properties of vWF, we adsorbed the platelet binding domain of vWF (A1 domain) to three surfaces: polystyrene, tissue culture polystyrene, and glass. Protein structure was investigated using x-ray photoelectron spectroscopy (XPS) and time of

flight secondary ion mass spectrometry (ToF-SIMS). Protein function was tested by measuring platelet binding in a physiologically relevant flow assay.

Using nitrogen as a marker of protein, XPS showed similar amounts of vWF A1 adsorbed to the three surfaces. However, the flow assay showed significantly different platelet binding to vWF A1 on each surface, as measured by platelet rolling velocity. Rolling velocity was highest on glass, indicating lowest platelet binding. The slowest rolling velocity was observed on polystyrene, indicating the highest level of platelet binding. ToF-SIMS data was analyzed using principle component analysis (PCA). PCA showed separation of the three surfaces with adsorbed vWF A1, indicating conformational differences between the proteins on each surface.

These studies show that surface properties influence structure and function of adsorbed vWF domains. Although there was a similar amount of protein on each surface, protein function was different. Polystyrene, the most hydrophobic of the surfaces, appeared to have the strongest activating effect on vWF. ToF-SIMS studies showed conformational differences, suggesting that conformational differences contribute to the observed functional differences.

Understanding the structure and function of adsorbed vWF gives insight into how vWF behaves on biomaterial surfaces and how this might affect platelet binding. Characterizing vWF adsorption also allows *in vitro* behavior to be more accurately related to *in vivo* thrombosis events.

Raghavachari. *Colloids Surf B* (2000) 19:315.

Kang. *Thromb Res* (2007) 119: 731.

5:00pm **BI-WeA10 Secondary Structures of Soft- and Reactively Landed Multiply Charged Protein Ions**, ***Q. Hu***, *P. Wang, J. Laskin*, Pacific Northwest National Laboratory

Soft- and reactive landing of mass-selected ions enables highly selective preparation of uniform thin films of a variety of complex molecules on surfaces. We previously demonstrated that conformationally-selected peptide arrays can be prepared using SL of peptide ions onto self-assembled monolayer (SAM) surfaces. In this work we studied the secondary structures of protein ions soft- and reactively landed onto SAM surfaces using infrared reflection absorption spectroscopy (IRRAS). Different charge states of ubiquitin were generated by electrospray ionization (ESI). The structure of the low charge state corresponds to the pseudo-native state of the protein and the high charge state corresponds to an unfolded state. Inert CH_3-terminated SAM (HSAM) and hydrophilic COOH-terminated SAM (COOH-SAM) were used as soft- landing targets. Detailed analysis of IRRAS spectra, especially the amide I band, provides valuable information on the secondary structures of the immobilized protein species. This technique allows us to study the effect of the initial conformation and the properties of the surface on the secondary structure of immobilized proteins. Secondary structure of ubiquitin ions reactively landed onto SAM of N-hydroxysuccinimidyl ester terminated alkylthiol on gold (NHS-SAM) was also studied by IRRAS, and the reaction rate was determined from the depletion of the strong asymmetric carbonyl stretching band of the NHS group.

5:20pm **BI-WeA11 Analysis of Unspecific Protein Adsorption onto Polymer Materials using Radioactive Labeling, Atomic Force Microscopy and ELISA**, ***M. Holmberg***, *X.L. Hou*, Technical University of Denmark

In this study radioactive labeling is used in combination with ELISA measurements and Atomic Force Microscopy (AFM) analysis to investigate aspects of unspecific protein adsorption onto polymer materials. The radioactive labeling is a setup in which different proteins are labeled with isotopes that emit gamma radiation with different energies. This makes it possible to detect several proteins simultaneously onto the same sample and thus to investigate competitive protein adsorption and how the presence of some proteins influence the adsorption of others. Results from protein adsorption onto polymer materials using the radioactive labeling setup have shown adsorption levels higher than expected for monolayer adsorption and suggest the existence of protein multilayers on some surfaces. Results from fibrinogen adsorption onto surfaces that are pre-adsorbed with albumin show that fibrinogen can adsorb on top of albumin and that exchange of already adsorbed albumin is not a dominant process during the competitive adsorption with fibrinogen. Preliminary results on QCM-D (quartz crystal microbalance with dissipation monitoring) also strengthen the idea of the existence of an interface between polymer surface and protein solution in which proteins interact with both each other and the surface in a matrix structure that have multilayer character. To further illustrate the impact on (or lack of) unspecific protein adsorption using blocking buffers and pre-adsorption of proteins, we show results from ELISA measurements of unspecific protein adsorption onto TCPS (tissue culture polystyrene) and PS (polystyrene). We can detect large difference in adsorption level between proteins with and without a HIS tag (six histidines) onto TCPS, but not onto PS, with the HIS tagged proteins showing much higher adsorption onto TCPS compared to the same protein without a HIS tag. Furthermore, low or none impact on the level of adsorption of these HIS tagged proteins is observed when the TCPS surfaces are blocked with BSA (bovine serum albumin). We are combining quantitative results from radioactive labeling (and QCM-D) with AFM analysis performed in liquid to obtain data regarding homogeneity and topography of adsorbed protein layers. Furthermore, ELISA is used as a supplementary technique to acquire more knowledge regarding unspecific adsorption of proteins onto polymer materials. The obtained information is of importance when evaluating interactions between proteins and biomaterials.

5:40pm **BI-WeA12 Surface Interactions of GG-X-GG and X_n Oligopeptides with Inorganic Substrates**, ***K.P. Fears***, *J.L. Kulp, T.D. Clark, D.Y. Petrovykh*, Naval Research Laboratory

The adsorption behavior of model GG-X-GG and X_n oligopeptides on Au and native Si oxide substrates was investigated to elucidate the contributions of different amino acids (AAs) to peptide-surface interactions. The manner in which peptides and proteins interact with surfaces is of critical importance in many biological and technological systems. The mechanisms underlying surface adsorption of proteins, however, are poorly understood, largely due to the inherent complexity of natural proteins. Accordingly, in this work simple model peptides were chosen to systematically examine the interactions between natural AAs and inorganic surfaces. Surface interactions of a series of AAs were probed by incubating inorganic substrates in aqueous solutions of model GG-X-GG pentapeptides, in which an AA of interest was flanked with Gly. The effects of cooperative adsorption were also examined using model X_n oligopeptides (n = 5, 10). The amount of peptides that *irreversibly* adsorbed on each substrate was quantified by X-ray photoelectron spectroscopy (XPS), the resulting systematic data revealed several trends in surface adsorption of oligopeptides as a function of their composition and length. On the negatively-charged, hydrophilic native SiO_x layer of a Si wafer, only peptides containing positively-charged residues (Lys and Arg) and polar residues (Ser and Thr) adsorbed at significant levels. Peptides adsorbed more readily on Au-coated Si wafers, on which the maximum surface coverage was ca. 3 times greater than that on the native SiO_x. For a particular AA (X), adsorption tended to increase, sometimes dramatically, with increasing units of X (GG-X-GG < X_5 < X_{10}). In pairs of AAs having side chains that only vary by alkyl chain length (L and V, Q and N, R and K, E and D), the AA with the longer alkyl chain adsorbed more readily, although this trend diminished with the increasing number of X residues.

Authors Index

Bold page numbers indicate the presenter