Wednesday Morning, October 20, 2010

Biomaterial Interfaces
Room: Taos - Session BI1-WeM

Biomolecules at Interfaces
Moderator: P. Kingshott, Aarhus University, Denmark

8:00am **BI1-WeM1 Lipid Membrane Interface Mediated Protein Misfolding and Aggregation**, ***E.Y. Chi***, University of New Mexico, *J. Majewski*, Los Alamos National Laboratory, *E. Mandelkow*, Max Planck Unit for Structural Molecular Biology, Germany, *K.Y. Lee*, University of Chicago

The misfolding and aggregation of the amyloid-beta (Ab) peptide and tau protein into fibrillar deposits are linked to the pathogenesis of Alzheimer's disease (AD). However, the molecular basis of the early events during the aggregation process and the nature of the structural fluctuations that triggers the misfolding and association of Ab and tau remain poorly understood. The lipid membrane interface has been implicated to mediate the fibrillogenesis of both proteins. Using model lipid membranes, we studied the nature and mode of lipid-protein interactions and characterized the effect of these interactions on the conformation and assembly of Ab and tau.

Both Ab and tau exhibit strong interactions with membranes composed of charged lipids, but interact weakly with zwitterionic lipids. To elucidate the molecular-scale structural details of Ab-membrane association, we used complementary X-ray and neutron scattering techniques (grazing-incidence X-ray diffraction, X-ray reflectivity, and neutron reflectivity) to investigate *in situ* the association of Ab with lipid monolayers at the air/water interface composed of either the negatively charged lipid DPPG, the zwitterionic lipid DPPC, or the cationic lipid DPTAP at the air/water interface. We found that the anionic lipid DPPG uniquely induced crystalline ordering of Ab at the membrane surface that closely mimicked the beta-sheet structures in fibrils, revealing an intriguing templated ordering effect of DPPG on Ab. Furthermore, incubating Ab with lipid vesicles containing the anionic lipid POPG induced the formation of amyloid fibrils, confirming that the templated ordering of Ab at the membrane surface seeded fibril formation. By measuring the interaction between different tau constructs (hTau40, K18 and K32) with membranes composed of different lipids, our data showed that tau's C-terminus, the microtubule binding domain, is responsible for its association with the lipid membrane. Moreover, hyperphophorylation which is an early and critical event in the pathogenesis of AD, as mimicked by a tau mutant, did not prevent tau from binding to lipid membrane.

Our study provides a detailed molecular-scale characterization of the early structural fluctuation and assembly events that may trigger the misfolding and aggregation of Ab *in vivo*. Our study suggests that the "soft", intrinsically unfolded nature of both Ab and tau can give rise to rich dynamic behaviors at interfaces, such as the lipid membrane interface. Our data implicate that the adsorption of Ab and tau to anionic lipids in the cell membrane may serve as an in vivo mechanism of templated aggregation and drive the pathogenesis of AD.

8:20am **BI1-WeM2 Effect of Metal Ions on Lipid Bilayer Formation on Semiconductor Surfaces**, ***R. Jain***, *A.J. Muscat*, University of Arizona

Lipid bilayers have applications in drug delivery, bio-sensing for clinical diagnosis, and device fabrication. Just as in a living cell where a lipid bilayer separates aqueous compartments from their surroundings, a lipid membrane supported on a surface can function as a mask that allows selective mass transport via intermembrane proteins. Lipid bilayers have been used primarily to support proton channel proteins in sensors, but there are many other types of intermembrane proteins with different functions. With an aim to extend the use of biomolecules in device fabrication, the effect of heavy metal ions on bilayer formation was investigated using atomic force microscopy (AFM) and x-ray photoelectron spectroscopy (XPS). 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) lipid molecules were used to form supported bilayers by the vesicle fusion method on hydroxylated Si, As_2O_3-terminated GaAs, and Al_2O_3 surfaces. Alumina was deposited on a p-Si(100) surface by atomic layer deposition (ALD) at 170°C using trimethylaluminum (TMA) and water as precursors. The bilayer was formed on an AFM stage for 6 hr and the stage was heated to 41.5±5°C, which is 17.5°C above the DMPC phase transition temperature. The height of lipid membranes measured with AFM after digging a hole was 4.9±0.5 nm on a hydroxylated Si surface and 4.0±0.6 nm on an alumina surface, which correspond to the thickness of a bilayer. The mechanical strength, uniformity, and integrity of membranes were measured after flowing copper sulfate solutions (100-3000 ppm) through a liquid flow cell over the formed bilayer on the Al_2O_3 surface. Al_2O_3 was chosen because Cu^{2+} ions are reduced to Cu^0 on alumina, but not on SiO_2. It was found by XPS that the copper permeates through the lipid bilayer and deposits on the alumina surface as Cu^0. Force distance measurements were made to understand copper permeation. The adhesion force of copper on Al_2O_3 was 2-3 times higher than that of lipid molecules, leading to breaking of the bilayer and deposition of copper. AFM confirmed the breakage and the bilayer thickness after copper exposure was 1.4±0.2 nm. This study shows that metal ions with a higher adhesion force than lipid molecules on an insulator surface disrupt bilayer formation, placing limitations on how bilayers can be used in device fabrication. These results also suggest an additional mechanism for the antibacterial properties of copper.

8:40am **BI1-WeM3 Characterizing Carbohydrate-Modified Surfaces: Advancing the Glycomics Paradigm**, ***D.M. Ratner***, University of Washington **INVITED**

Carbohydrates and glycoconjugates are involved in a myriad of biological processes, including fertility, cancer, the immune response, and host-pathogen interactions. The carbohydrate microarray (or glycoarray) has emerged as one of the most promising technologies capable of revealing the complex roles played by carbohydrates in biology and medicine. While the glycoarray has had a significant impact on the field of glycomics (the study of carbohydrates in biology), little is known about the function of surface chemistry on array performance. In addition, existing glycoarray technologies are non-standardized, utilize disparate chemistries, and are only partially optimized to interrogate low affinity interactions or discriminate between mono- and multivalent binding. To help realize the glycoarray's full potential in glycomics research, new label-free and high-throughput diagnostic tools are needed to screen glycan-dependent interactions and expand our fundamental understanding of glycobiology. The reengineered glycoarray must also include a quantitative picture of glycan surface chemistry to advance our ability to match array results with the biological question or hypothesis being tested.

This study details the development a panel of carbohydrate-functionalized ultrasensitive label-free biosensors based on surface plasmon resonance imaging (SPRi), novel silicon photonic devices, and microelectrode microarrays. To define the role of biosensor surface chemistries, we describe the application of advanced surface analytical techniques to exhaustively characterize the biointerface of carbohydrate-modified surfaces for biosensor applications. X-Ray Photoelectron Spectroscopy (XPS), Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS), and Scanning Probe Microscopy (SPM) are used to examine the chemistries of glycoarray surfaces to establish the relationship between biosensor performance and glycan surface density and molecular conformation—highlighting the contributions of the surface analysis and biointerfaces communities in advancing the nascent field of glycomics.

9:20am **BI1-WeM5 *In situ* Characterization of DNA Films**, ***C. Howell***, ***P. Koelsch***, University of Heidelberg and Karlsruhe Institute of Technology, Germany

Films of thymine, adenine and cytosine single-stranded DNA (homo-oligonucleotides) immobilized on gold were characterized in liquid under various conditions using broadband sum-frequency-generation (SFG) spectroscopy. Spectra of the three film types under these *in situ* conditions in the C-H stretching region were significantly different than those obtained in air and appeared to show unique contributions based on the nucleobase composition of the film. This could be the result of base-specific hydration differences for these films. Data in air showed no identifiable unique base contributions in this region; however, significant differences in peak intensities among the three film types were evident in the spectra. These differences appeared to correlate with the degree of order of the films, suggesting that SFG spectroscopy can be useful for detecting overall order in these types of systems. Hybridization behavior of these systems was also studied, with results showing detectable differences between hybridized and unhybridized films. These results contribute to the understanding of DNA films and help to establish a foundation for the investigation of more complex biomolecules *in situ* using SFG spectroscopy.

9:40am **BI1-WeM6 Surface Characterization of Mixed DNA/Mercaptoundecanol Assembly on Gold**, ***N. Vandencasteele***, *L. Árnadóttir, J.E. Baio, T.M. Weidner, L.J. Gamble*, University of Washington

The hybridization efficiency of DNA microarrays and biosensors is determined in part by variables such as the density and orientation of the single stranded DNA oligomers used to build the devices. In order to better understand the chemistry on the microarray surfaces, and therefore improve their response, we study model surfaces of DNA adsorbed on gold with x-

ray photoelectron spectroscopy (XPS), near-edge x-ray absorption fine structure (NEXAFS) spectroscopy and time-of-flight secondary ion mass spectrometry (ToF-SIMS) to characterize the surface order and structure. We previously showed that varying the amount of diluent molecule, and thus the probe density, affects the hybridization efficiency of a 20mer thiolated single stranded DNA probe and the target.[1,2] In this study we compare the density and orientation of a 40mer single-stranded thiolated DNA (HS-ss-DNA), when varying amounts of the diluent molecule 11-mercapto-1-undecanol (MCU) are adsorbed. DNA is detected on the surface using XPS (N1s and P2p peaks) and ToF-SIMS analysis. ToF-SIMS results for various backfill times and various backfilling media are compared using PCA analysis. In the first set of experiments the MCU backfill reaction takes place in water (ultra pure 18 MΩ), in the second set of experiment it takes place in sodium Tris EDTA buffer solution (STE). We showed that when the co-adsorption reaction is done with a solution of MCU in water, the amount of MCU that incorporates into the surface saturates at 18h and the amount of DNA on the surface remains relatively constant. When the same experiment is carried out using a MCU solution in STE buffer the MCU binding removes the surface bound DNA and all of the HS-ss-DNA is removed from the surface after 2h, suggesting a better hydration of the DNA in the STE medium. The improved hydration of the DNA increases it's mobility in the solution allowing an easier access of the MCU to the gold surface. Angle dependent NEXAFS spectra taken for DNA layers after different MCU backfill times in water showed the highest degree of orientational order after a 30 min MCU backfilling step. The data also indicate that the DNA nucleotide base rings are ordered parallel to the surface. Hybridization efficiencies of the 40 mer DNA layer with various densities will be compared with surface plasmon resonance (SPR).

(1) Gong, P.; Lee, C.-Y.; Gamble, L. J.; Castner, D. G.; Grainger, D. W. ***Analytical Chemistry*** **2006,** ***78*****, 3326-3334.**

(2) Lee, C.-Y.; Gong, P.; Harbers, G. M.; Grainger, D. W.; Castner, D. G.; Gamble, L. J. ***Analytical Chemistry*** **2006,** ***78*****, 3316-3325.**

Authors Index

Bold page numbers indicate the presenter

— A —

Árnadóttir, L.: BI1-WeM6, 1

— B —

Baio, J.E.: BI1-WeM6, 1

— C —

Chi, E.Y.: BI1-WeM1, **1**

— G —

Gamble, L.J.: BI1-WeM6, 1

— H —

Howell, C.: BI1-WeM5, **1**

— J —

Jain, R.: BI1-WeM2, **1**

— K —

Koelsch, P.: BI1-WeM5, 1

— L —

Lee, K.Y.: BI1-WeM1, 1

— M —

Majewski, J.: BI1-WeM1, 1
Mandelkow, E.: BI1-WeM1, 1
Muscat, A.J.: BI1-WeM2, 1

— R —

Ratner, D.M.: BI1-WeM3, **1**

— V —

Vandencasteele, N.: BI1-WeM6, **1**

— W —

Weidner, T.M.: BI1-WeM6, 1