Biomaterial Interfaces
Room 2014 - Session BI-FrM

Biomolecular Surface Characterization II

Moderator: P. Kingshott, The Interdisclinary Nanoscience Centre (iNANO), Denmark

8:00am **BI-FrM1 Probing Relations Between Molecular Orientation and Electron Transfer in Immobilized Metalloprotein Films Using Frequency-Domain, Planar Waveguide Spectroelectrochemistry**, ***S. Saavedra***, *Z. Oraci, A.F. Runge, W.J. Doherty, S.B. Mendes,* University of Arizona **INVITED**

This talk will describe efforts to develop a better understanding of the relationship between structure of a protein film immobilized on an electrode surface and its electrochemical activity, which is a prerequisite to design of protein-based molecular devices in which vectorial, heterogeneous electron transfer is required for efficient operation. The relationship between molecular orientation and electron transfer in immobilized films of redox-active proteins is being investigated using planar waveguide spectroelectrochemistry. This approach has been used to determine the porphyrin tilt angle distribution in a cytochrome c submonolayer adsorbed to an indium-tin oxide (ITO) electrode. However, only about half of the film is electroactive, which makes to difficult to correlate the broad orientation distribution (measured spectroscopically on the entire film) with the electron transfer rate (measured electrochemically on the electroactive portion of the film). To address this problem, a novel form of electroreflectance spectroscopy, potential-modulated, attenuated total reflection spectroscopy (PM-ATR), has been developed. In PM-ATR, the waveguide output is monitored while an ac potential modulation is simultaneously applied to the planar waveguide electrode. Changes in the absorbance as a function of the light polarization, modulation frequency, and amplitude provide information about electron transfer rates, electro-optical switching rates, and molecular orientation. For cytochrome c films on ITO, the electron transfer rate measured using TM polarized light was four-fold greater than that measured using TE polarized light, which is consistent with a shorter tunneling distance for molecules adsorbed in a vertical orientation (probed with TM) vs. molecules adsorbed in a horizontal orientation (probed with TE). These data are the first to correlate a distribution of molecular orientations with a distribution of electron transfer rates in a redox-active molecular film.

8:40am **BI-FrM3 Volumetric Interpretation of Protein Adsorption: Partition Coefficients, Interphase Volumes, and Free Energies of Adsorption to Hydrophobic Surfaces**, *E. Vogler,* ***H. Noh***, Penn State University

Interpretive mass-balance equations were derived from a model premised on the idea that protein reversibly partitions from bulk solution into a three-dimensional (3D) interphase volume separating the physical adsorbent surface from bulk solution. Theory was shown to both anticipate and accommodate adsorption of all proteins to the two test surfaces, suggesting that the underlying model was descriptive of the essential physical chemistry of protein adsorption. Application of mass balance equations to experimental data permitted quantification of partition coefficients P, interphase volumes V,@footnote 1@ and the number of hypothetical layers M occupied by protein adsorbed within V.@footnote 2@ Partition coefficients measure the equilibrium ratio of interphase and bulk-solution-phase w/v (mg/mL) concentrations W@footnote 3@ and W,@footnote 4@ respectively, such that P-W@footnote 5@/W.@footnote 6@ Proteins studied were found to be weak biosurfactants with 45< P <520 and commensurately low apparent free-energy-of-adsorption, (-6RT<@footnote 7@G@footnote 8@=-RTlnP<-4RT). These measurements corroborate independent estimates obtained from interfacial energetics of adsorption (tensiometry) and are in agreement with thermochemical measurements for related proteins by hydrophobic-interaction chromatography. Proteins with molecular weight MW<100kDa were found to occupy a single layer at surface saturation whereas the larger proteins IgG and Fib required two layers. @FootnoteText@ @footnote 1@sub I@footnote 2@sub I@footnote 3@sub I@footnote 4@sub B@footnote 5@sub I@footnote 6@sub B@footnote 7@DELTA@footnote 8@sub ads.

9:00am **BI-FrM4 Adsorption-Induced Changes in Protein Bioactivity Correlated with Adsorbed Protein Orientation and Conformation**, ***K.P. Fears***, *R.A. Latour,* Clemson University

It is well accepted that an irreversibly bound adsorbed protein layer forms on biomaterial surfaces very rapidly after they come in contact with bodily fluids. The structure and bioactivity of this adsorbed protein layer are recognized to be critical factors that influence subsequent cellular responses; however, little is known about the actual molecular mechanisms involved. The bioactivity of an adsorbed protein could be inhibited due to adsorption-induced conformational changes in the proteinâ?Ts structure or due to orientational effects that result from the active site being sterically blocked by the surface. A set of experimental methods have been developed using alkanethiol self-assembled monolayers (SAMs), with different surface chemistries, along with surface plasmon resonance (SPR) spectroscopy to measure the adsorption-induced changes in protein bioactivity using enzymes with well known molecular structures. The secondary structure of the adsorbed protein layers was determined using circular dichroism (CD) and compared to the native structure of the proteins. Molecular models of the proteins showing the location of hydrophobic and charged residues, as well as the active site residues, were analyzed along with the data collected on adsorbed protein bioactivity and secondary structure to identify the most likely cause for the measured changes in bioactivity as a function of surface chemistry.

9:20am **BI-FrM5 SIMS Detection of Peptides on Alkanethiol Self-Assembled Monolayers**, ***Z. Zhu***, *O. Hadjar, P. Wang, J. Laskin,* Pacific Northwest National Laboratory

Alkanethiol self-assembled monolayers (SAM) on Au substrate provide a very ordered and controllable surface which is friendly to bio-molecules. Numerous protein or DNA molecules have been immobilized on alkanethiol SAMs to fabricate bio-active surfaces which can be used for bio-analysis or some other applications. A number of techniques, such as fluorescence, X-ray photoelectron spectroscopy (XPS), reflection IR, surface plasmon resonance, have been applied to detect these bio-active molecules immobilized on alkanethiol SAM surfaces. During the last several years, secondary ion mass spectrometry (SIMS) detection of bio-active molecules has been of great interest, and a number of efforts have been reported. Although several papers have addressed on SIMS detection of bio-active molecules on alkanethiol SAM surfaces, the detection limit of this technique is not clear. Since static SIMS is a very surface-specific technique, sample preparation plays a very important role in such research. In this work, the electrospray technique was used to prepare samples due to its effectiveness, easy control and simple operation. Sub-monolayer of three peptides (Bradykinin, MW=1060.5; Gramicidin S, MW=1141.6; Substance P, MW=1347.7) were prepared on three alkanethiol SAMs (-S(CH2)11CH3, -S(CH2)10CO2H, -S(CH2)2(CF2)9CF3) and detection limits were tested. 15 keV Ga+ ions were used as the primary ion source. It has been found that the detection limit of peptide molecules can be as low as 0.0001 monolayer or even lower. Our results show that SIMS is a very promising technique to characterize low-mass protein molecules on alkanethiol SAM surfaces.

9:40am **BI-FrM6 Temperature Stability of Protein Monolayers Studied by Ellipsometry in the Infrared, Visible and Ultraviolet Spectral Regions**, ***H. Arwin***, Linköping University, Sweden*; D.W. Thompson, J.A. Woollam,* University of Nebraska, Lincoln

Future devices based on bionanotechnology may contain protein layers and temperature stability will be an issue. One way to monitor temperature induced structural changes in protein molecules adsorbed in monolayers on solid substrates is to analyze the complex-valued refractive index N=n+ik as determined by spectroscopic ellipsometry. In the infrared spectral (IR) region ellipsometry provides a quantification of the amide band parameters of the adsorbed protein molecules and in the visible (VIS) and ultraviolet (UV) regions the layer thickness (surface mass density) can be determined as well as the electronic contributions to N. Earlier studies on protein multilayers (human serum albumin and its antibody) show that heating above 100 °C causes structural changes observed as changes of the amide bands. Heating to 200 °C causes layer degradation seen as irreversible changes in n and k and also a substantial decrease in surface mass density. The objective is here to present methodology and results from pilot studies of effects of heating monolayers of proteins adsorbed on gold substrates. The surface mass density and N=n+ik are determined with spectroscopic ellipsometry (UV-VIS-IR) equipped with a heat stage (20 - 300 °C). A model refractive index function is applied to the optical properties of the protein layer and changes in the model parameters are monitored at elevated temperatures with special emphasis on the amide I band around 1640 cm@super -1@, the amide II band around 1520 cm@super -1@ and the amide A band in the 2800 - 3300 cm@super -1@ region.

10:00am **BI-FrM7 Fibronectin Adsorption onto Tantalum-Oxide Examined by QCM-D and Elipsometry: The Influence of Nano-Roughness**, ***M.B. Hovgaard,*** University of Aarhus, Denmark, DK*; K. Rechendorff, F. Besenbacher, M. Foss,* University of Aarhus, Denmark

The adsorption of fibronectin onto tantalum-oxide is investigated probing the effects of a nano-rough, stochastic morphology on the structure and functionality of an adsorbed protein layer. Nano-rough substrates were produced by e-gun evaporation of tantalum onto standard QCM-D substrates at oblique incidence deposition, allowing the production of self-affine surfaces@footnote 1@ with ~ 4 nm RMS roughness as characterized by AFM. A comparative study of the protein adsorption onto flat versus nano-rough substrates was conducted using Ellipsometry and QCM-D. A characterization of the adsorbed layers was facilitated by variation of the protein concentration and monitoring of the specific versus non-specific antibody adsorption along with utilizing the different sensitivity of the two techniques.This including a direct comparison between the dry(Ellipsometry) and hydrated(QCM-D) surface densities further extended by the viscoelastic properties(QCM-D) of the adsorbed layers. Results show a significant influence of the nano-roughness on saturation coverage and the viscoelastic properties of the resulting protein layer. Compared to the flat morphology, the saturated protein films on the rough substrates show higher surface densities (area-effect) and non-trivial effects such as a more rigid packing (lower QCM-D dissipation) and decreased specific antibody adsorption, both indicating a changed protein configuration. @FootnoteText@ @footnote 1@ K. Rechendorff, M.B.Hovgaard, M.Foss, F.Besenbacher Appl. Phys. Lett. 87, 073105 (2005).

10:20am **BI-FrM8 Electrodeposition of Chitosan for BioMEMS: Real-Time, In-Vitro, and Post-Process Characterization**, ***S.L. Beatty****, E.C. Dreyer, X. Luo, G. Rubloff,* University of Maryland

Chitosan has been shown to serve as a robust and reproducible scaffold for biological functionalization in microfluidic channels, using chitosan electrodeposition at specific bioMEMS sites. To better understand the materials and process science of chitosan electrodeposition onto metal electrodes, we have used both in vitro characterization techniques and post-deposition measurements of air-dried films. Real-time current, voltage, and optical reflectivity show film growth nonlinearities expected for electrical vs. optical property sensing. Raman spectroscopy of films air-dried after electrodeposition reveals the presence of primary amine groups active in biofunctionalization. AFM images of the air-dried films reveal variable and rough morphology not directly correlated to deposition conditions, while hydration increased surface homogeneity. Fluorescence microscopy using fluorescently decorated chitosan shows both film growth and its spatial distribution across the deposition electrode, enabling a comparison of in-vitro and dry morphology of the chitosan film. While the in-vitro images show fairly smooth distribution of chitosan, the air-dried films are much rougher, indicating nonuniform and unpredictable collapse of the film's structure during drying. Thus, in vitro measurements of the film deposition and structure are essential to exploit the potential of chitosan as a platform for biotechnology applications.

10:40am **BI-FrM9 Biomolecule Adsorption Studies at Interfaces using Sum Frequency Generation Vibrational Spectroscopy, Quartz Crystal Microbalance, Dielectric Spectroscopy, and Conducting-Probe Atomic Force Microscopy**, ***O. Mermut****, R.L. York, D.C. Phillips, J.Y. Park,* UC, Berkeley and Lawrence Berkeley National Lab*; K.R. McCrea, R.S. Ward,* Polymer Technology Group*; G.A. Somorjai,* UC, Berkeley and Lawrence Berkeley National Lab

A combination of different surface-specific techniques (probing macroscopic and molecular-scale properties) was employed to examine the adsorption behavior of biomolecules onto surfaces of variable chemistry and hydrophobicity. Various short-chain model peptides, composed to two types of amino acids (hydrophilic and hydrophobic) were synthesized to investigate the effect of the amino acid side-chain chemistry on adsorption at the water/polystyrene and the water/silica interface. Specifically, these peptides contain hydrophobic (X) and charged (Y) amino acids with sequence: Ac-XYYXXYXXYYXXYX-NH@sub 2@ (designed to yield an alpha-helical peptide) or Ac-XYXYXYX-NH@sub 2@ (designed to yield a beta-strand peptide). Sum Frequency Generation (SFG), a non-linear optical spectroscopic tool, provided molecular-level information regarding the orientation of the adsorbed biomolecules. Specifically, the hydrophobic amino acid residues preferentially order on hydrophobic polystyrene/water interfaces. This ordering is strongly dependent on chain length and sequence. Quartz Crystal Microbalance (QCM) allowed quantitative in situ determination of the adsorbed mass of material, which is influenced by the surface hydrophobicity. Impedance measurements were obtained using Dielectric Spectroscopy to investigate perturbations of the electrical double layer in the presence of adsorbed biomaterial. Lastly, we measured the morphological properties (topography, aggregation), mechanical properties (adhesion and friction force), and electrical transport properties (conductance) of adsorbed peptide at these various liquid-solid interfaces using a Conducting Probe-Atomic Force Microscopy (CP-AFM). Using this combinatorial approach of techniques, we will discuss how molecular level information correlates with macromolecular properties.

11:00am **BI-FrM10 Low-Fouling Amine-Terminated Poly(ethylene Glycol) Thin Layers and Effect of Immobilization Conditions on Their Mechanical and Physicochemical Properties**, ***Y. Martin****, P. Vermette,* Université de Sherbrooke, Canada

The physicochemical and mechanical properties of amine-terminated covalently-bound poly(ethylene glycol) (PEG) layers are dependent on fabrication methods. Particularly, the use of a theta solvent yields dense bound PEG layers with properties not well described by traditional models. The polymer concentration is also known to be important for the layer properties. In this study, NHS-PEG-tBoc molecules of molecular weight 3,400 were immobilized on plasma-generated primary amine-containing surfaces at different concentrations and using different solvents including theta solvents. Light diffraction techniques were used in an attempt to understand the influence of polymer aggregation kinetics in a theta solvent on the final properties of the fabricated PEG layers. The polymer layer properties were characterized using X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM) in force mode, quartz crystal microbalance (QCM) and fluorescence microscopy. Results show that polymer concentration in solution is an important indicator of final layer properties, and that the use of a theta solvent induces complex aggregation phenomena in solution, dependant on PEG concentration, yielding layers with unique properties such as greatly variable low-fouling potential or water trapping attributes. The PEG layers fabricated through the process described in this article are also shown to be chemically reactive, paving the way for the immobilization of bio-active molecules.

11:20am **BI-FrM11 Single Molecule Force Spectroscopy on 5'-Methyl thioadenosine/S-Adenosylhomocysteine Nucleosidase (MTAN) from Escherichia Coli by Atomic Force Microscopy**, ***B.I. Kim****, J.O. Holmes, K.A. Cornell,* Boise State University*; P. Deschatelets,* Potentia Pharmaceuticals Inc.

5'-Methyl Thioadenosine/S-Adenosylhomocysteine Nucleosidase (MTAN) is a dual substrate specific enzyme that catabolizes 5'Methyl thioadenosine (MTA) or S-Adenosylhomocysteine (SAH) to form adenine and 5'-methylthioribose or S-ribosylhomocysteine, respectively, in many pathogenic microbes. MTAN is an ideal target for new antibiotic development because it has no corresponding human equivalent and recognizes a substrate that is not present in mammalian cells. Based on this interest, the binding mechanisms of various transition state analogues that inhibit the expression of MTAN were studied using the single molecule force-spectroscopy technique. Force-distance curves were measured as a function of separation distance between a probing molecule and the MTAN molecule under buffer conditions using atomic force microscopy (AFM). Various probing molecules were covalently linked via a flexible spacer polyethylene glycol (PEG) to the tip of an AFM. The force-distance curve exhibits an unbinding event in the retracting curve with a certain unbinding force. Single molecular binding strength, rate constant, and structural data for the binding pocket were extracted from several hundred force-distance curves and were analyzed statistically for each transition state analogue. The statistical values were compared with the equilibrium dissociation constants previously obtained by other groups. Single molecule force spectroscopy provides a new insight into the specific binding mechanism between the inhibitors and a MTAN molecule at the single molecular level.

11:40am **BI-FrM12 Single-Molecule Force Spectroscopy of Stimulus-Responsive Polypeptides**, ***S. Zauscher****, A. Valiaev, A. Chilkoti, T. Oas,* Duke University

Stimulus-responsive elastin-like polypeptides (ELPs) experience a large entropic collapse when exposed to an environmental stimulus, such as an increase in temperature. While interfacial applications of ELPs have been prototypically demonstrated, a systematic investigation of the phase transition behavior at the solid-liquid interface and on the single-molecule level is lacking. We will present results from single molecule force-spectroscopy (SMFS) measurements probing the force-extension and conformational behavior of ELPs, below and above their transition temperature. We show that ELPs are well described by a random coil polymer model, suggesting the absence of significant secondary structure.

Furthermore, we show that single molecule force spectroscopy is able to differentiate different ELP constructs by distinguishing differences in the hydrophobic hydration of side chains. This suggests that SMFS has potential diagnostic abilities for studying the hydration behavior of proteins. We also noticed that some ELP force-extension curves showed temperature independent deviations that could not be described by polymer elasticity models developed for random polymers. We argue that the observed deviations arise from the force-induced cis-trans isomerization of prolines, which are repeated every fifth residue in the main chain of ELPs. We present evidence for this mechanism by Monte Carlo simulations of the force-extension curves using an elastically coupled two-level system. Furthermore, we show results from control experiments with poly-L-proline that demonstrate the similarity of the conformational transition between poly-L-proline and ELPs. We believe that our work is the first demonstration of force induced cis-trans isomerization in proline containing polypeptides. Our results suggest that SMFS could be used to assay proline cis-trans isomerization in proteins and may thus have significant potential diagnostic utility.

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