Biomaterial Interfaces
Room 210D - Session BI-MoM

In-Situ Spectroscopy of Biomolecules at Interface

Moderator: M. Grunze, University of Heidelberg, Germany

8:20am **BI-MoM1 High-Resolution Structural and Dynamic Characterization of Proteins on Biomaterial Surfaces**, ***P.S. Stayton***, *G.P. Drobny,* University of Washington **INVITED**

The development of materials with bioactive interfaces is a major focus of the biomaterials and tissue engineering communities. There is also considerable interest in the immobilization of active peptides and proteins in separations, diagnostics, proteomics, and cell culture technologies. In order to design appropriate biomaterial modification strategies where activity is retained, it is desirable to elucidate how protein structure, dynamics and orientation are related to the biomaterial surface properties and immobilization chemistries. Solid-state NMR techniques provide an opportunity to determine these molecular structure and dynamics properties of proteins and peptides on many different types of biomaterial surfaces. In particular, the high-resolution backbone conformation of proteins can be determined, the binding "footprint" - or which amino acid side-chains actually contact the surface can be determined, the role of water at the protein-material interface can be investigated, the dynamics of specific protein side-chains can be determined, and the orientation of the protein on the crystal surface can be determined. Homonuclear and heteronuclear dipolar recoupling solid-state NMR techniques, combined with dynamic studies, have been applied to determine the structure, dynamics and orientation of proteins and peptides immobilized on polymeric biomaterial surfaces, surface-modified nanoparticles, and on inorganic crystals such as hydroxyapatite.

9:00am **BI-MoM3 Protein-Surface Interactions Studied with Internal Reflection Ellipsometry**, ***H. Arwin***, *M. Poksinski,* Linköping University, Sweden

Spectroscopic ellipsometry (SE) used in internal reflection mode exhibits very large sensitivity for in situ protein adsorption on thin metal layers if used at surface plasmon (SP) resonance conditions@footnote 1@. Compared to external SE, the protein layer induced changes in the ellipsometric parameter @DELTA@ are several orders of magnitude larger. Using this high sensitivity it becomes possible to extract more details than only protein surface mass density (or film thickness) from SE data. In situ determination of the microstructure of adsorbed protein layers, e. g. in terms of mass distribution perpendicular to a surface, is within reach. Another implication is an increased sensitivity in biosensor applications. The enhanced sensitivity is here verified experimentally with adsorption studies of human serum albumin on gold and the possibility to model protein layer microstructure from SE data is demonstrated. Access to such detailed information is of relevance to understand conformation, surface interaction, dynamics and function of proteins at interfaces. The increased sensitivity is discussed in a thin film approximation of the complex reflectance ratio. It is found that the @DELTA@-sensitivity is inversely proportional to the difference between the damping @GAMMA@ of an SP if the metal is semi-infinite and the change in damping due to that the metal film is thin. The SE sensitivity is thus in principle unlimited as the metal-film induced change in damping can be selected with the film thickness and made to match @GAMMA@. However, the sensitivity becomes finite due to non-idealities of the sample, beam divergence, finite bandwidth of the light, etc. @FootnoteText@ 1. H Arwin, M Poksinski and K Johansen, Total internal reflection ellipsometry: principles and applications, Appl Opt, in press; M Poksinski and H Arwin, Protein monolayers monitored by internal reflection ellipsometry, Thin Solid Films 455-456 (2004) 716-721.

9:20am **BI-MoM4 Angle-resolved Imaging Surface Plasmon Resonance**, *D.A. Armitage,* The University of Nottingham, UK; ***P.M. Williams***, The University of Nottingham, UK, U.K.

Surface plasmon resonance (SPR) has evolved in recent years into a commercially recognized technique for analyzing surface interactions with a film thickness resolution on the sub-nanometer scale. SPR imaging can also be employed to obtain data on the spatial distribution of molecules at surfaces. However, the instrumentation currently used in SPR imaging experiments has reduced capabilities for precise SPR angle and hence thickness and refractive index measurements compared to conventional non-imaging systems. By combining a non-imaging SPR system with a micro-positioning stage we demonstrate that a 2-D image of SPR response can be produced whilst retaining high precision angle sensitivity.

9:40am **BI-MoM5 Feasibility Study of a Waveguide Excitation Fluorescence Microscope for Micro and Nanoscale Characterization of Bio-Interfaces**, ***H.M. Grandin***, *B. Städler, J. Vörös, M. Textor,* Swiss Federal Institute of Technology (ETH), Switzerland

The ability to investigate the interactions that occur between a biological system and a surface, be it a native biological surface or a synthetic surface, is of critical importance to our fundamental understanding of biomaterials and their many applications in biosensors, medical implants, and tissue engineering. Our development of a Waveguide Excitation Fluorescence Microscope (WExFM) satisfies this need uniquely by providing a means for the quantitative study of bio-interfacial interactions in-situ, e.g.: protein adsorption and cell adhesion, with both temporal and spatial resolution. Although other techniques are capable of either quantitative studies, e.g.: optical waveguide lightmode spectroscopy, or of spatially resolved imaging at the interface, e.g.: total internal reflection fluorescence microscopy, the WExFM is the only technique currently available which can provide both simultaneously. Further advantages include high target sensitivity for fluorescence detection (femtoMolar range) and high surface specificity (ca. 100 nm perpendicular to the waveguide), as well as, the capability to perform multicolour imaging, large area analysis with submicron resolution, and 'built-in' calibration of fluorescent light gain. Preliminary results from streptavidin-biotin binding studies have been obtained with sub-picoMolar sensitivity, thus, demonstrating the feasibility of this technique. In this presentation the principles and experimental set-up of the WExFM will be introduced, potential applications for in-situ, real-time quantitative monitoring of protein- and cell-surface interactions will be discussed and finally, first results demonstrating the feasibility of the WExFM will be presented.

10:00am **BI-MoM6 In situ Sum Frequency Generation Characterization of Peptide Monolayers on Hydrophobic and Charged Surfaces**, ***N.T. Samuel***, University of Washington; *K. McCrea,* The Polymer Technology Group; *L.J. Gamble,* University of Washington; *R.S. Ward,* The Polymer Technology Group; *P.S. Stayton,* University of Washington; *G.A. Somorjai,* University of California at Berkeley; *D.G. Castner,* University of Washington

Immobilization of bioactive peptides is an active research area for diagnostics, cell culture and biomedical implants. Previous studies have shown well-defined sequences of lysine (K) and leucine (L) containing peptides spontaneously adsorb onto hydrophobic substrates with either @alpha@-helix or @beta@-sheet secondary structures. In this study the adsorption of these peptides onto hydrophobic and charged surfaces has been characterized in situ with IR-Visible Sum Frequency Generation (SFG) spectroscopy. The SFG spectra in the CH, NH and OH stretch regions show the adsorption of the LK peptides onto these substrates is mediated by interactions through their leucine (hydrophobic surfaces) and lysine (charged surfaces) residues. These hydrophobic and electrostatic interactions are accompanied by ordering of the functional groups involved in the interaction. Ordering of water molecules at these interfaces is also observed. SFG spectra in the amide I region were used to examine the secondary structure of the LK peptides. For the @alpha@-helix LK peptide the @alpha@-helix secondary structure is maintained upon dehydration of the sample, even though significant changes in the side chain ordering was observed. Polarization-dependent Near-edge X-ray Absorption Fine Structure (NEXAFS) experiments were also done on the adsorbed peptides. The results demonstrate that the N1s->@pi@*@sub CONH@ feature in the Nitrogen K-edge is sensitive to the secondary structure of the adsorbed peptide. NEXAFS experiments also confirm the highly ordered nature of the adsorbed peptides.

10:20am **BI-MoM7 Measurement of Conformational Changes of Surface Bound Biomolecules: a Novel Strategy for Analytical Biosensing**, ***D.A. Russell***, *L.M. May,* University of East Anglia, UK

A large number of biomolecules change conformation upon interaction with specific substrates. Whilst spectroscopic techniques (such as CD, NMR and IR) provide sensitive measurement of secondary structure in solution, they are not amenable for the development of surface bound sensing technologies based on analyte induced conformational changes. Surface plasmon resonance (SPR) is a surface sensitive technique capable of measuring changes in refractive index (RI) that occur in proximity to the sensor interface. By depositing biomolecules onto the gold-coated sensor surface of an SPR instrument it is possible to measure changes of secondary structural conformation as a function of substrate concentration. A number of biomolecules including, polypeptides, proteins

and enzymes, have been formulated as SAMs on SPR sensor surfaces and varying concentrations of substrates or mild denaturants have been passed over the monolayer surface to elicit secondary structural changes. For example, a dramatic increase in the SPR signal (m°) was observed when polylysine was induced into the alpha-helical conformation with ethanol. Similarly, the SPR signal was related to other secondary structures (including beta-sheet and random configurations) of both polypeptides and the protein Concanavalin A. The intensity of the SPR signal being related to the RI of the secondary structural configuration of the biomolecule. Development of this sensing strategy has focused on the self-assembly of urease in order to measure the conformational change of this enzyme as a function of heavy metals. On the sensor surface, the SPR signal from the urease monolayer linearly increased in intensity as a function of cadmium concentration in the range 0 - 10 mg/L. These data show that conformational changes of surface bound biomolecules can be measured and used analytically.

10:40am **BI-MoM8 Study of Metal Film-Tethered (Bio)Molecules in Aqueous Solution by Enhanced ATR-IR Spectroscopy**, ***D.P. Land***, *J.S. Toofan, C.M. Gerth,* University of California, Davis

Data are presented illustrating the application of overlayer-enhanced attenuated transmission-Fourier transform infrared spectroscopy (E-ATR-FTIR). The presence of a thin metal film at in internal reflection interface accomplishes several important feats. It enhances and concentrates the electric field in the near-surface region. It introduces a wide range of chemical possibilities for surface modifications by which analytes can likewise be concentrated near the interface. And it minimizes interactions with the bulk solvent, facilitating the use of IR spectroscopy in aqueous (and other) solutions. The combination of effects facilitates the study of numerous solution systems, and most importantly, perhaps, aqueous solutions. The experiment has been modeled to reveal details of the dependence upon prism material (ZnSe and Ge), thin film thickness and composition (metals and oxides), as well as angle of incidence and adsorbate properties. Experimentally, several key experiments have been performed. Data will be shown which includes the study of reactions of thiols with the gold films, in aqueous and other solutions. Additionally, spectra for tethered proteins, antibodies, and protein-antibody reactions have already been acquired and will be presented. Detection limits in the tens of femtomoles for proteins and antibodies has been achieved. Experiments currently underway include reactions of enzymes with tethered inhibitors and of tethered enzymes with substrates and co-factors.

11:00am **BI-MoM9 Investigation of Fibrinogen Displacement from Oxide Surfaces**, ***P.S. Cremer,*** Texas A&M University **INVITED**

This talk will discuss the adsorption and displacement of fibrinogen at the silica/aqueous interface. It has been known since Leo Vroman's original studies in 1969 that fibrinogen is one of the first proteins to adsorb from human plasma on oxide surfaces, but it is ultimately displaced by other smaller and less abundant species in solution. We have employed a combination of vibrational sum frequency spectroscopy (VSFS), atomic force microscopy, immunoassays, and kinetic studies to unravel the molecular level details of the mechanism for this process. The results reveal that lysine and arginine residues on the protein's alpha-C domains interact with the surface via weak electrostatic binding. The rest of the protein can only make stronger hydrogen bonding and hydrophobic contacts once these domains have been displaced. In particular, the VSFS data give direct evidence for alignment of arginine and lysine residues with the surface in the protein's most displaceable configuration.

11:40am **BI-MoM11 Probing the Conformation of Hydrated Molecular Adsorbates on Solid Interfaces Using Long Period X-ray Standing Wave Fluorescence**, ***C.A. Crot***, *C. Wu, M. Schlossman,* University of Illinois at Chicago*; T.P. Trainor,* University of Alaska*; P.J. Eng,* University of Chicago*; L. Hanley,* University of Illinois at Chicago

Understanding the process of protein and biomolecular adsorption onto solid surfaces is of great importance in a wide variety of applications including biomaterials, tissue engineering, biosensors, immunoassays, and protein arrays. However, direct investigation of adsorption processes and the hydrated conformation of a molecular adsorbate is difficult since the majority of surface analysis techniques require ultra-high vacuum conditions. In this work long period x-ray standing wave fluorescence spectroscopy (XSW) is being developed as a spatial probe of molecular adsorption at the liquid-solid interface using a model surface-adsorbate system. A 25 nm thick polystyrene layer is spin coated on a thick silicon wafer, then the top of this layer is amine-functionalized via hyperthermal allyl amine ion deposition. X-ray photoelectron spectroscopy and atomic force microscopy are used to monitor the chemistry and morphology of this amine-polystyrene model surface. A thirteen residue peptide is covalently bound to a poly(ethyleneglycol) chain that is terminated with a bromine labeled amino acid and used as the model adsorbate. This Br-PEG-peptide construct is adsorbed onto the amine surface and its hydrated conformation is examined by XSW and x-ray reflectivity. Measurements of the bromine fluorescent yield as a function of incident angle provides information on the distance of the bromine layer from the silicon surface with an accuracy of several angstroms. Preliminary data analysis of the Br-PEG-peptide conformation indicates the peptide end is adsorbing directly onto the amine surface while the bromine atom on the Br-PEG end is extended ~13@+-@3 nm from the amine surface into the aqueous layer. Adsorbate configuration is probed as a function of adsorption time, amine film characteristics, and other experimental parameters. The general applicability of the XSW technique to probe the conformation of labeled adsorbates at the aqueous-solid interface is discussed.

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