Monday Afternoon, November 4, 2002

Biomaterials
Room: C-201 - Session BI+VT-MoA

Protein Surface Interactions
Moderator: D. Grainger, Colorado State University

2:00pm **BI+VT-MoA1 Self-Assembled Monolayers of Carboxy-Terminated Poly(ethylene glycol): Protein Resistance, Biospecific Functionalization and Application to Immunodiagnostics**, ***R. Dahint***, University of Heidelberg, Germany **INVITED**

The high specifity of antigen/antibody reactions has been widely exploited to develop accurate detection methods for biomolecules. Heterogeneous immunoassays, where proteins are selectively bound by immobilized antibodies and detected by the use of labeled secondary antibodies are a standard diagnostic technique. Also, a considerable amount of research has been focused on immunosensor development. A general problem in immunodiagnostics is non-specific protein adsorption: Macromolecules are not only bound to the substrate by specific antigen/antibody recognition, but also adhere due to non-specific interaction forces. Hereby, the accurate determination of antigen concentration may be significantly deteriorated. Moreover, non-specifically adsorbed proteins may even block and deactivate the immobilized receptors. The integration of specific receptors into a protein resistant matrix would, therefore, significantly improve quantitative analysis. Self-assembled monolayers (SAMs) of poly- and oligo(ethylene glycol) have proven to effectively prevent protein adsorption. We, therefore, synthesized a carboxy-terminated poly(ethylene glycol) alkanethiol ($HOOC\text{-}CH_2\text{-}(OCH_2\text{-}CH_2)_n\text{-}O\text{-}(CH_2)_{11}\text{-}SH$, n = 22-45) which facilitates covalent coupling of antibodies. In contrast to most other previous studies, where receptors have been coupled to SAMs formed from a binary mixture of differently functionalized molecules, only a single chemical functionality is involved. After characterizing the films by infrared absorption (FTIR) and X-ray photoelectron spectroscopy, ellipsometry and contact angle measurements, their performance as bioselective coatings with reduced non-specific adsorption has been tested in both FTIR and acoustic wave sensor experiments. The protein resistant properties of the films are put in context with previous results on oligo(ethylene glycol) alkanethiolate SAMs including neutron reflectivity studies on protein/surface interactions.

2:40pm **BI+VT-MoA3 ToF-SIMS and XPS Analysis of Enzymatic Digests of Adsorbed Protein Films**, ***M.S. Wagner***, *D.G. Castner*, University of Washington

Characterization of multicomponent adsorbed protein films is critical in understanding biological interactions with surfaces. We have previously shown that Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) can quantify the composition of binary and ternary adsorbed protein films using the low mass ($0 < m/z < 200$) fragmentation pattern of the mass spectrum. However, quantification of more complex protein films using this method is limited to the most abundant proteins present in the films. Therefore, trypsin digestion of the adsorbed proteins was performed to generate tryptic fragments for subsequent analysis by ToF-SIMS. The tryptic fragments were identified by combining ToF-SIMS with matrices from Matrix Assisted Laser Desorption and Ionization Mass Spectrometry (MALDI-MS). Residual protein remaining on the substrate after digestion was detected using ToF-SIMS and X-ray Photoelectron Spectroscopy (XPS). This method provides unique fragments for the identification of adsorbed proteins by ToF-SIMS.

3:00pm **BI+VT-MoA4 Protein Adsorption on Colloidal Oxide Particles**, ***K. Rezwan***, *L.P. Meier, M. Textor, L.J. Gauckler*, ETH Zurich, Switzerland

Protein adsorption to surfaces of medical implants is an essential aspect of the cascade of biological reactions taking place at the interface between synthetic material and biological environment. The types and amounts of adsorbed proteins mediate subsequent adhesion, proliferation and differentiation of cells as well as deposition of mineral phases. Most metallic biomaterials are covered by a protective, stable oxide film such as titanium oxide on titanium. Hence proteins only interact with the oxide film and not with the underlying metal. Closer investigations of the protein - oxide interface are therefore of great relevance to the biomaterials field. In the past, protein adsorption and desorption has been investigated mostly on planar surfaces by in situ techniques such as ellipsometry, optical waveguide lightmode spectroscopy (OWLS) and quartz crystal microbalance (QCM). The drawback of these methods is the lack of direct information about surface charges, which are known to strongly affect protein adhesion and conformation at interfaces. We used colloid chemistry analysis methods such as colloidal vibration potential (CVP), X - ray disc centrifuge (XDC) measurements and UV â€“ spectroscopy (UVS) to study in detail the adsorption of proteins to well-defined colloidal particles of typically 100 - 200 nm diameter. Combining these methods, the adsorbed amount of proteins and its influence on the zetapotential and the isoelectric point of the particles were determined with great precision and across a wide pH range. Adsorption of bovine serum albumin was found to alter the zetapotential of the oxides Al2O3, TiO2 and SiO2 and their isoelectric points to an extent that depended on the adsorbed mass. Combining UVS and XDC, the volume density and the thickness of the protein layer could be determined. The thickness corresponded to a monolayer or less. The adsorbed mass of albumin turned out to be nearly independent of pH in the range from pH 2 to 12.

3:20pm **BI+VT-MoA5 Prediction of Adsorption Behavior of Fibronectin as a Function of Surface Functionality Using a Customized Protein Adsorption Force-Field**, ***R.A. Latour***, *K.A. Wilson*, Clemson University, *A.J. Garcia*, Georgia Institute of Technology, *S.J. Stuart*, Clemson University

The ability of a cell to bind to an adsorbed protein layer on a biomaterial surface is dependent on the structure and availability of the protein's cell binding domains following adsorption. A well-known example of this is integrin binding to the PHSRN and RGD sites located on the 9th & 10th type III repeats of fibronectin (Fn). The objective of this research was to utilize computational chemistry to predict the relative orientation and accessibility of these cell-binding domains in Fn after adsorption as a function of surface functionality (CH3, OH, NH3+, COO-). Modeling was conducted using an SGI O2/Onyx computational system with InsightII software (Accelrys). The Charmm force-field was used to simulate intramolecular interactions for the fibronectin, while a new set of force-field parameters was created to simulate the interactions between the fibronectin and the surface. The new force-field parameters were set to provide similar energy vs. surface separation plots for peptide residue-surface adsorption as determined by previous semi-empirical modeling studies using MOPAC/PM3/COSMO. Initial energy vs Fn orientation maps were generated followed by 50 ps molecular dynamics simulations at selected positions to assess initial adsorbed Fn behavior. Results suggest that the CH3 and COO- surfaces should most strongly inhibit integrin binding, but by different mechanisms; the CH3 surface by disrupting Fn structure and the COO- surface by blocking accessibility. The OH and NH3 surfaces were predicted to preserve binding site structure and accessibility. Results compare favorably with experimental studies and provide likely molecular mechanisms that help explain experimentally observed behavior.

3:40pm **BI+VT-MoA6 Analysis of Organic and Biological Materials in Ultra-High Vacuum**, ***D.G. Castner***, University of Washington **INVITED**

Ultra-high vacuum (UHV) surface science has a long, successful history in the fields of catalysis and microelectronics. The early adaptation of UHV-based tools in these fields was largely due to the fact that the materials involved (metals, ceramics, semiconductors, etc.) were readily vacuum compatible. This talk will address the challenges of adapting UHV surface analysis techniques for analyzing organic and biological materials. These include their higher vapor pressure, their increased susceptibility to X-ray, electron, and ion sample degradation, and vacuum induced changes in their structure. Some of the first organic surface analysis experiments were done on polymers. Since that time, these experiments have been extended to self-assembled monolayers (SAMs), biomaterials, and adsorbed biomolecules. Examples to be discussed from these areas will include the effect of polymer additives, surface rearrangement of polymers, the well-defined structure of SAMs, and preserving the conformation of adsorbed proteins.

4:20pm **BI+VT-MoA8 PEG-ylated Surfaces with Graded Protein Interactiveness : A ToF-SIMS, XPS and Optical Waveguide Sensor Study**, ***S. Pasche***, *S.M. De Paul, J. Vörös*, Swiss Federal Institute of Technology, *P. Hug, B. Keller*, Swiss Federal Laboratory for Material Testing and Research, *H.J. Griesser*, University of South Australia, *N.D. Spencer, M. Textor*, Swiss Federal Institute of Technology

Poly(L-lysine) grafted with poly(ethylene glycol) (PLL-g-PEG), a polycationic co-polymer positively charged at neutral pH, has been shown to spontaneously adsorb onto negatively charged surfaces, rendering them protein-resistant to a degree related to the PEG surface density. Since the PEG surface density is a function of polymer architecture (PEG molecular weight and grafting ratio expressed as number of lysine monomers per PEG side chain), it becomes feasible to control the interactiveness of a surface by varying the co-polymer architecture. Angle-dependent XPS and ToF-SIMS

were used to investigate the surface-chemical properties. The adsorbed mass after serum exposure was determined by an optical sensor technique. Further colloid-modified AFM force measurements aim at studying the mechanical properties of the coated surfaces. PLL-g-PEG was adsorbed onto niobium oxide coated wafers, resulting in the formation of stable polymeric monolayers. The grafting ratio, g, of the polymer was varied systematically between 2 and 10, leading, upon surface adsorption, to highly different, but controlled PEG surface densities. PEG molecular weight was varied between 1000 and 5000. Polymer adsorbed mass was determined quantitatively by an in situ optical waveguide technique. A quantitative relationship was established between EG-monomer surface density, calculated from the known polymer architecture and the surface-adsorbed mass, ToF-SIMS intensities of PEG-, PLL- and substrate-related secondary ion peaks, and the amount of serum proteins that adsorbed onto the different polymer-coated surfaces. PLL-g-PEG surface-coating technology allows the fabrication of surfaces with tailored interactiveness and the establishment of design criteria for PEG-based, protein-resistant surfaces.

4:40pm **BI+VT-MoA9 Time-of-Flight Secondary Ion Mass Spectrometry Analysis of Conformational Changes in Adsorbed Protein Films**, ***N. Xia***, University of Washington, *C.J. May*, Yale University, *S.L. McArthur*, *D.G. Castner*, University of Washington

Characterizing the identity, composition, conformation, and orientation of adsorbed proteins is essential for the development of biocompatible devices. Static time-of-flight secondary mass spectrometry (ToF-SIMS) is a powerful surface analytical technique for analyzing adsorbed protein films. However, the ToF-SIMS experiment is done under vacuum, and drying adsorbed proteins for analysis can denature or change their conformation. In this study, trehalose coating was used to inhibit these conformational changes from occurring during sample preparation for ToF-SIMS analysis. Surface plasmon resonance (SPR) analysis showed that air-dried films of trehalose-stabilized antibodies retained a significant proportion of their hydrated antigen binding activity. In contrast, air-drying without trehalose protection resulted in the adsorbed protein films losing most of their antigen binding activity. Structural differences between trehalose-stabilized and unstabilized protein films were then analyzed with static ToF-SIMS. By application of principle component analysis (PCA) to the ToF-SIMS spectra, the biological activity difference observed in SPR was correlated to changes in protein conformation. Trehalose-protected proteins retained a greater degree of their original conformation than the unprotected proteins. This suggests that static ToF-SIMS has the capability to distinguish conformational differences in adsorbed protein films. Moreover, trehalose protection can be used for static ToF-SIMS analysis of adsorbed protein films to obtain structural information that is more relevant to the structure of the proteins in aqueous conditions.

5:00pm **BI+VT-MoA10 Study of the Adsorption Kinetics and Conformational Changes of Human Serum Albumin and Human Plasma Fibronectin using PM-RAIRS, Radiolabelling and Atomic Force Microscopy**, ***R.J. Manning***, *C.M.J. Fauroux*, *M.J. Pilling*, *P. Gardner*, *G.J. Leggett*, University of Manchester Institute of Science and Technology, UK

The kinetics of adsorption of proteins has been studied on self assembled monolayers (SAMs) on gold, formed by the adsorption of alkanethiolates with differing functional groups and varying alkyl chain lengths. The adsorption of human serum albumin (HSA) and human plasma fibronectin (HPF) has been studied using three complementary techniques: post modulation fourier transform reflection adsorption infrared spectroscopy (PM-RAIRS), radiolabelling, and atomic force microscopy (AFM). Initial adsorption kinetics of HSA and HPF were established using FTIR. It was found that monolayer coverage was reached faster on methyl terminated SAMs than on hydroxyl and carboxylic acid terminated hydrophilic monolayers. Tritium radiolabelling of HSA and HPF confirmed the trends observed with FTIR. The conformations of the adsorbed proteins were followed using PM-RAIRS, enabling quantitative monitoring of the percentage of α-helix, β-sheet, β-turn and random coils, indicating the degree of denaturation on differing surfaces over time. Finally, AFM was used to generate direct observations of layers of adsorbed proteins, providing useful insights into the distribution of proteins across the differing surfaces and enabling individual molecules to be observed. HSA was found to form a fibrillar network on methyl terminated SAMs at low concentrations and short adsorption times, whilst individual molecules were observed on hydroxyl and carboxylic acid terminated monolayers. This study demonstrates the complimentarity of FTIR, radiolabelling and AFM in understanding the adsorption of proteins on well-ordered SAMs.

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