Biomaterial Interfaces
Room 311 - Session BI1-WeM

Protein-Surface Interactions
Moderator: M. Textor, ETH Zurich, Switzerland

8:40am **BI1-WeM2 Functionalization of Diamond and Silicon Surfaces with Molecular Monolayers to Control Protein-Surface Interactions**, *T. Lasseter Clare, B. Clare, N. Abbott, B.M. Nichols,* ***R.J. Hamers,*** University of Wisconsin-Madison

We have investigated the chemical functionalization of diamond and silicon surfaces with short ethylene glycol (EG) oligomers to control the the nonspecific adsorption of proteins to these surfaces. EG oligomers bearing a terminal vinyl group were linked to H-terminated surfaces of diamond and silicon using illumination with ultraviolet light at 254 nm. The resulting layers were characterized by XPS, and the effects of EG oligomers on binding of avidin, casein, fibrinogen, and BSA were qualitatively investigated using on-chip fluorescence measurements. To avoid issues related to fluorescence quenching and facilitate quantitative comparison on different materials, we measured avidin adsorption using an elution method. These experiments show that EG-modified surfaces of nanocrysalline diamond, single-crystal silicon, and polycrystalline gold films all resist binding of avidin to nearly the same extent. One of the attractive features of diamond is its extraordinary chemical stability. In a comparison of EG-modified surfaces of diamond, silicon, and gold, we find that gold and silicon samples undergo a significant degradation over a time period of approximately 1 week, while EG-diamond samples undergo no detectable change. These results are corraborated with XPS measurements that show silicon and gold undergo partial loss of their functionalization layers, while EG-diamond shows no measurable change. The effects of surface roughness were investigated by comparing EG-modified surfaces of nanocrystalline, polished, and cleaved single-crystal diamond. The influence of monolayer termination and other factors will also be presented. Overall, these measurements show that photochemical modification of Si and diamond with vinyl-terminated EG oligomers is a very effective way to reduce nonspecific adsorption. They also provide new molecular insights into the factors the control protein adsorption at surfaces.

9:00am **BI1-WeM3 The QCM-D Technique for Control of Protein Binding on Nanoscale LSPR Active Substrates**, ***F. Höök,*** Lund University, Sweden **INVITED**

In the search for surface modifications that minimizes the influence on the structure and function of adsorbed proteins, supported phospholipid bilayers (SPBs) have been proven inert towards protein adsorption from as complex mixtures as serum. Since they at the same time fulfils the requirements set on specific coupling of both water-soluble and membrane bound proteins, have made them attractive in various biosensor applications and as coatings for biomaterials. However, so far limited progress has been made with respect to SPB formation on nanoscale solid supports. By utilizing insights gained from quartz crystal microbalance with dissipation (QCM-D) monitoring of protein/lipid interactions on either Au or SiO2, we have established a surface-modification protocol that enables localized rupture of phospholipid vesicles on SiO2 in the bottom of nanometric holes in a thin Au film. The hole-induced localization of the localized surface plasmon resonance (LSPR) field to the voids of the holes is demonstrated to provide a novel concept for studies of protein-binding reactions confined exclusively to SPB-patches supported on SiO2.@footnote 1@ In particular, we emphasize the possibility to in this way perform label-free studies of lipid-membrane mediated reaction kinetics, including the compatibility of the assay with array-based recording, with signals originating from bound protein in the sub-zeptomole regime. Extensions of this concept includes the use of the conductive LSPR hole substrate (i) as one of the electrodes of the QCM-D sensors, enabling simultaneous QCM-D and LSPR readouts of reactions occurring on, for example, either Au or SiO2, and (ii) for studies of protein binding to individual colloidal particles that match the size of the holes. @FootnoteText@ @footnote 1@Svedhem S, Pfeiffer I, Larsson C, Wingren C, Borrebaeck C, Höök F. ChemBioChem 2003:339-343. @footnote 2@Dahlin A, Zach M, Rindzevicius T, Kall M, Sutherland DS, Hook F. JACS 2005, 127:5043-5048.

9:40am **BI1-WeM5 In Deuterated Water the Unspecific Adsorption of Proteins Is Significantly Slowed Down: Results of an SPR-study using Model Organic Surfaces**, ***Chr. Grunwald,*** Ruhr-University, Germany; *J. Kuhlmann,* Max-Planck-Institute for Molecular Physiology, Germany; *Ch. Woell,* Ruhr-University, Germany

The control of unspecific adsorption of proteins to natural and technical surfaces plays an important role in biology and also for many applications. Organic model surfaces e.g. self-assembled monolayers, are often used to identify fundamental surface and/or protein properties that rule protein adsorption.@footnote 1@ Some techniques involved in biointerface research require the use of heavy water, e.g. neutron scattering techniques.@footnote 2@ Also in NMR studies D@sub 2@O is the solvent of choice when focusing on biomolecular and hydration dynamics. So far several studies have been concerned with the characterization of the unspecific adsorption of proteins from normal water buffers.@footnote 3@ In the present work we report a comparison of the unspecific protein adsorption from normal and heavy water buffers. Previously it has been assumed that the surface kinetic of the unspecific adsorption is unaffected by the substitution of water by D@sub 2@O.@footnote 2@ However, for the four proteins investigated here this assumption does not hold. The ratio k@sub H@/k@sub D@ of the adsorption rate constants of the different buffer conditions describe the strength of the isotope effect. We have measured ratios between 1.0 and 2.6 indicating that the adsorption kinetics are strongly affected by a H@sub 2@O-D@sub 2@O-substitution. @FootnoteText@ @footnote 1@Herrwerth, S.; Eck, W.; Reinhardt, S.; Grunze, M. Journal of the American Chemical Society 2003, 125, 9359-9366.@footnote 2@Schwendel, D.; Hayashi, T.; Dahint, R.; Pertsin, A.; Grunze, M.; Steitz, R.; Schreiber, F. Langmuir 2003, 19, 2284-2293.@footnote 3@Ostuni, E.; Grzybowski, B. A.; Mrksich, M.; Roberts, C. S.; Whitesides, G. M. Langmuir 2003, 19, 1861-1872.

10:00am **BI1-WeM6 The Development of Molecular Simulation Capabilities as a Tool to Understand Protein Adsorption Behavior at the Molecular Level**, *F. Wang, Y. Sun, S.J. Stuart,* ***R.A. Latour,*** Clemson University

Although important, the molecular mechanisms involved in protein adsorption processes are still not well understood. Empirical force field-based molecular simulation methods have been successfully developed to enable molecular mechanisms to be studied for other applications, such as protein folding and ligand-receptor binding; these methods have similar potential to help elucidate the molecular mechanisms for protein adsorption. Two of the most important problems that must be addressed before methods can be developed for this application are the force field problem and the sampling problem. The force field problem relates to the design of the energy function and its parameters that control how atoms interact with one another during a simulation. The sampling problem relates to the need to sample molecular events over timeframes that extend far beyond those that are capable of being reached using standard molecular dynamics methods. The objective of our research is to develop computational methods to address both of these issues, with an initial focus on the development of methods to calculate the free energy of peptide adsorption. In conventional simulations, peptides become stuck in low-energy conformations and this prevents adsorption free energy from being accurately calculated. We are therefore developing biased-sampling methods to enable adequate conformational space to be sampled in peptide-surface simulations so that adsorption free energy can be properly calculated. With this capability, the accuracy of a protein adsorption force field can be evaluated, modified, and validated by comparison between calculated adsorption free energy and experimentally measured values.

10:20am **BI1-WeM7 Model Dielectric Functions for Adsorbed Protein Layers**, ***H. Arwin,*** Linköping University, Sweden; *J.A. Woollam, D.W. Thompson,* University of Nebraska, Lincoln

A detailed knowledge about protein-surface interactions is of crucial importance for development of biomaterials, bioanalytical tools and biosensors as well as for understanding the mechanisms in protein-cell interactions. Ellipsometry is extensively used in these areas due to its nm-resolution in layer thickness and capability for in situ studies at solid-liquid interfaces. The outputs from an ellipsometer study are typically quantification of adsorbed surface mass and/or dynamics of protein adsorption. Recently also infrared ellipsometry has become available and optical signatures like amide bands in surface-bound proteins can be quantified. However, for layers of nm thickness, the analysis is not straight forward and it can be hard to separate thickness and refractive index of the protein layer, especially with single wavelength ellipsometry data.

Spectroscopic ellipsometry can in some cases resolve this but very few studies are reported. Another major advantage if spectroscopic data are available is the possibility to model, i.e. to parameterize the wavelength dispersion of the refractive index. This also leads to noise reduction. In this report a model dielectric function (MDF) concept for protein layers is proposed. Notice that the square root of the MDF equals the refractive index. The MDF is based on a Cauchy dispersion to which Lorentzian and/or Gaussian resonances are added to account for electronic and vibrational excitations in ultraviolet/visible and infrared spectral regions, respectively. The use of the proposed MDF is exemplified on fibrinogen adsorbed on gold. With variable angle spectroscopic ellipsometry, data were recorded in the spectral region 0.2 to 30 µm (300-5000 cm@super-1@) before and after protein adsorption. In the analysis the fibrinogen layer thickness is obtained as well as the refractive index (the square root of an MDF) and the amide bands I, II and A are resolved. A generalization to protein layers in general will be discussed.

10:40am **BI1-WeM8 Protein Nanopatterning onto Nanostructured Polymer Surfaces**, ***C. Satriano***, *G.M.L. Messina, G. Marletta,* University of Catania, Italy

The preferential adsorption of human fibronectin, lactoferrin, serum albumin and lysozime has been investigated onto nanostructured polysiloxane surfaces obtained by a colloidal crystal-based technique in combination with cold plasma treatment. In particular, 2D arrays of nanopores, having typical dimensions of about 55 nm of diameter and about 3 nm and 1 nm respectively for the rim height and the pore depth were fabricated. Polystyrene nanoparticles were used to imprint regularly-spaced nanopores within a bilayer formed by an untreated polysiloxane film onto a plasma-modified one. The internal area of the pores consisted of hydrophilic O@sub 2@-plasma treated polysiloxane, while the external surface was the hydrophobic untreated polymer. The spatially-resolved features of adsorbed proteins were investigated by means of Atomic Force Microscopy. The in situ adsorption process on homogeneously modified surfaces has been investigated by means of Quartz Crystal Microbalance with Dissipation Monitoring, while X-ray Photoelectron Spectroscopy was employed to evaluate ex-situ the coverage and thickness of the protein adlayers for the two types of surfaces. The results showed that the relevant chemical factors are the surface free energy and the chemical termination of the different surfaces. In particular, human fibronectin and lactoferrin showed a preferential adsorption outside of the hydrophilic nanopores, while lysozime and human serum albumin seem prefer the nanopore area. The results suggest that it could be possible to achieve the separation of protein mixtures by a spatially resolved adsorption.

11:00am **BI1-WeM9 Immobilization of Protein Nano-Clusters on Polymeric Nano-Craters**, *A. Valsesia,* ***P. Colpo***, EC-JRC-IHCP Italy*; M. Lejeune,* EC-JRC-IHCP Italy, Italy*; F. Bretagnol, T. Meziani,* EC-JRC-IHCP Italy*; F. Rossi,* EC-JRC-IHCP Italy, Italy

The reduction of the typical length scale in the creation of patterned surfaces is of high interest in the field of bio-interacting materials and more particularly for biosensors design. For instance, the creation of sub-micrometric or nano-metric patterns is important for the miniaturization of the actual protein and DNA micro-spotting technology, or for the minimization of the non-specific absorption in biosensors, or to increase the orientation capability of immunosensors. To generate these patterned surfaces at the submicron level, the use of classical optical lithography methods becomes complex, since they are reaching the diffraction limits when the feature sizes are lower than 200 nm. Among the alternative techniques of lithography such as E-beam, nano-sphere lithography is a reliable method to produce nano-topography over large area surfaces. In this work we developed a reliable technique to produce polymeric nano-craters with bio-specific carboxylic functionalities and with controlled surface density and distribution, surrounded by an homogeneous matrix of anti fouling polymer. The process has been carried out combining plasma deposition and etching techniques with colloidal masking. The plasma etching process parameters have to be accurately studied in order to create the nano-structures without affecting their chemical properties. The micro-structural characterization of the nano-structured films was carried out by the combination of Ellipsometry, FT-IR spectroscopy and Atomic Force Microscopy (AFM). The surface chemical contrast at the nano-scale was characterized by using Chemical Force Microscopy. The creation of nano-patterned surfaces with controlled topography and chemistry at the sub-micron scale was demonstrated. In particular a contrast in the wettability between the two nano-regions was observed. The preferential absorption of the biomolecules inside the fouling nano-craters was demonstrated by Confocal Fluorescence Microscopy measurements.

11:20am **BI1-WeM10 Binding of the Streptococcal C5a Peptidase to Immobilized Fibronectin**, ***J.R. Hull***, University of Washington*; G. Tamura,* The University of Washington Dept. of Pediatrics*; D.G. Castner,* University of Washington

Group B Streptococci (GBS) are a leading cause of sepsis and meningitis in newborns, and an emerging cause of serious bacterial infections in immunocompromised adults and the elderly. The streptococcal C5a peptidase (ScpB) of GBS is found in virtually all clinical isolates of GBS. ScpB inhibits neutrophil chemotaxis by enzymatically cleaving the complement component C5a. ScpB is a known Fibronectin (Fn) adhesin; however, it only binds to immobilized Fn and not soluble Fn. Therefore, it is unknown whether or not ScpB binds to a conformational determinate of Fn or multiple adjacent Fn molecules. For this study, surface plasmon resonance (SPR) was used to determine the affinity of ScpB for immobilized Fn. The measured affinity is in the nM range, which is biologically significant. ScpB was tethered to an atomic force microscope (AFM) tip via the bifunctional cross linker pyridyldithio poly(ethylene glycol) succinimidylpropionate (NHS-PEG-PDP). Each step of the tip functionalization was verified by X-ray photoelectron spectroscopy, static secondary ion mass spectrometry, and infrared spectroscopy. Adsorbed Fn was imaged via intermittent contact AFM with the ScpB modified tip at varying surface concentrations. Then force-distance curves were used to measure the interactions between ScpB and adsorbed Fn.

11:40am **BI1-WeM11 Enzyme Adsorption as a Model System to Probe Adsorption-Induced Changes in Protein Bioactivity**, ***K.P. Fears***, *Y. Sun, R.A. Latour,* Clemson University

Although the control of the bioactivity of adsorbed proteins is recognized to be critical for the control of cellular response, little is known about the actual molecular mechanisms involved. Molecular simulation provides great potential to elucidate these mechanisms and to be developed as a tool for surface design to control the orientation, conformation, and bioactivity of adsorbed proteins. The development of accurate molecular simulation methods, however, is critically dependent on the development of experimental methods that can be used to isolate specific molecular events using protein-surface systems that are sufficiently simple to enable them to be represented in molecular simulations. The objective of this research is to experimentally develop model enzyme adsorption systems for this purpose. Homogenous alkanethiol self-assembled monolayers with various end group functionalities are being used in conjunction with surface plasmon resonance spectroscopy to measure the effect of adsorption on protein bioactivity using a set of small enzymes (e.g, lysozyme, trypsin) with known molecular structure, bioactive site, substrate, and native-state bioactivity. An adsorbed trypsin layer on a positively charged surface was measured to be approximately 96% active, only 5% active on a hydrophobic surface, and have no detected activity on a negatively charged surface. It is hypothesized that orientational and conformational effects are primarily responsible for the differences between the charged surfaces and the hydrophobic surface, respectively. Circular dichroism studies are planned to measure the secondary structures of the adsorbed proteins to support this hypothesis.

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