Biomaterial Interfaces
Room 2014 - Session BI-WeM

Bio-interfacial Modification and Bio-Immobilization I (Honoring Marcus Textor, ETH-Zürich for Substantial Contributions to the Field)

Moderator: A. Chilkoti, Duke University

8:00am **BI-WeM1 Switchable BioInterfaces with Nanoscale Control**, ***J. Vörös,*** Lab of Biosensors and Bioelectronics, Switzerland*; C.S. Tang,* Swiss Federal Labs for Materials Testing and Res.*; C. Huwiler, B. Stadler, T. Blattler, M. Halter, M. Bally, M. Gabi, D. Grieshaber, O. Guillaume-Gentil,* Lab of Biosensors and Bioelectronics, Switzerland **INVITED**

The success of biomaterials critically depends on the ability to interact with the biological environment. The bioresponse is often determined by the properties of the biointerface which requires precise control on the micron- and nanometer scale. This presentation will highlight examples on how current state-of-the-art surface modification methods, such as self-assembled monolayers and poly(ethylene glycol) grafted polyelectrolytes can be used to tailor biointerfaces. For both molecular systems, functional groups are directly introduced into the molecule. Such functional groups are shown to have three advantages: a) they enable production of well-defined and stable surfaces; b) they can be functionalized with biologically relevant reactive groups such as capture probes, antibodies, peptides, drugs or growth factors; and c) they can be combined with (nano)lithography techniques producing patterns with different surface chemistries on the submicron scale.@footnote 1@ Engineered surfaces with such control are ideal platforms for sensing applications (e.g. for microarrays, or for bioaffinity sensors) and at the same time they can also be applied to drug delivery systems and biomaterials, to control specific and non-specific biomolecule - surface interactions. Recently, we have put a lot of efforts into achieving not only spatial but also a dynamic control over the properties of biointerfaces. Surfaces that change upon external stimuli provide us with new research tools for studying complex biological systems and to overcome difficulties in producing heterogeneous microarrays of fragile biomolecules. Highlights for the use of novel, electronically@footnote 2@ - or photo-active@footnote 3@ surfaces for applications in biosensing and local drug delivery will be presented. @FootnoteText@ @footnote 1@ J. Voros et al; MRS Bulletin, 30(3):202-206, 2005.@footnote 2@ C.S. Tang et al; Analytical Chemistry, 78:711-717, 2006.@footnote 3@ B. Stadler et al; Langmuir, 20: 11348-11354, 2004.

8:40am **BI-WeM3 Investigation and Quantification of Immobilized Protein in Crosslinked Bilayers**, ***R. Michel,*** University of Washington*; V. Subramaniam,* University of Arizona*; S.L. McArthur,* University of Sheffield, UK*; E. Ross, S. Saavedra,* University of Arizona*; D.G. Castner,* University of Washington

Supported lipid bilayers are commonly used as model systems for cell studies. More recently, these layers, which are inherently unstable under ambient or ultra-high vacuum conditions, have been stabilized via cross-linking. These crosslinked bilayers have potential applications as biomedical coatings and biosensors. The detection and investigation of intercalating biomolecules is a first step towards creating artificial cell-like surfaces, and proteins are ideal model systems to study functionality in the bilayer. Recently it was shown that a common membrane protein, rhodopsin, could be reconstituted into these lipid bilayers and retain its photoactivity after cross-linking of the lipid bilayers. Secondary Ion Mass Spectrometry (SIMS) and X-Ray Photoelectron Spectroscopy (XPS) are surface sensitive techniques that yield information on the top few nanometers. We used these ultrahigh vacuum techniques to structurally characterize the lipid bilayers of redox and UV polymerized supported lipid membranes composed of 1,2-bis[10-(2',4'-hexadienoloxy)decanoyl]-sn-glycero-3-phosphocholine (bis-Sorb PC). UV-polymerized bis-Sorb PC bilayers with reconstituted rhodopsin were quantified with XPS. Angle resolved XPS revealed the protein to be located within the bilayer, and not adsorbed on top of it. SIMS was used to investigate the similarities and differences of distinct fragments from the phosphocholine lipids and the protein amino acids.

9:00am **BI-WeM4 Proteo-Phospholipid Assemblies on Nanotextured Surfaces**, ***G.P. Lopez,*** University of New Mexico

This talk will describe our recent efforts to develop methods for incorporation of transmembrane proteins, peptides and receptors in new types of lipid bilayer assemblies supported on nanotextured substrates. Substrates investigated include those fabricated by synthetic (i.e., bottom-up) methods based, for example, on molecular self-assembly and reductive (top-down) nanolithographic methods. Several methods for incorporating tethered receptors, transmembrane proteins and peptides into phospholipids architectures will be presented and methods for characterizing these assemblies will be discussed. The potential for such supported and suspended bilayer assemblies in biotechnological applications will also be suggested.

9:20am **BI-WeM5 Transcending the Time-Domain in Protein Adsorption Simulations**, ***R.A. Latour,*** *Y. Sun,* Clemson University

Although very important, the ability to predict and control the bioactive state of adsorbed or tethered proteins remains a major challenge in the field of biomaterials. While molecular modeling methods have great potential to help understand protein-surface interactions, methods must be specifically developed for this application. Two of the most important problems that must be addressed are the force field problem and the sampling problem. The force field problem relates to the design of the energy function and its parameters to ensure that atoms of a given system interact with one another in a realistic manner 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 this research is to develop computational methods to address both of these issues, with a focus on the application of an advanced sampling method called replica-exchange molecular dynamics (REMD). An REMD simulation transcends the time-domain problem by enabling a Boltzmann-weighted ensemble of states to be generated, thus providing simulation results that should be directly comparable to experimental results for an equilibrated system. In this study, REMD simulations have been conducted to simulate lysozyme adsorption on a hydrophobic surface using a modified CHARMM force field with implicit solvation. The REMD simulations predict five different predominant configurations of lysozyme when adsorbed on this surface. Matched experimental studies are currently being conducted to enable the accuracy of the simulation results to be assessed.

9:40am **BI-WeM6 Biofunctionalizing Nitride Surfaces without Silanes**, ***R. Stine,*** Naval Research Laboratory, US*; K.M. McCoy, S.P. Mulvaney, L.J. Whitman,* Naval Research Laboratory

Silicon nitride is widely used as a coating in the microelectronics industry because of its ability to resist penetration by contaminants such as water, oxygen, and ionic species. This property also makes silicon nitride a common terminal passivation layer for chip-based biosensors and bioMEMS devices, all of which come into contact with aqueous saline solutions. Current methods for biofunctionalizing silicon nitride rely almost exclusively on silane-based films, both for direct functionalization and as bifunctional linkers. However, even under stringent controls, the chemistry of silane films on silicon nitride surfaces is notoriously inconsistent and suffers from degradation over time when used in aqueous environments. We have developed an alternate, silane-free, method for functionalizing silicon nitride surfaces. The native oxide is first stripped via HF solution, and then treated with a plasma that makes the surface reactive to aldehydes. Using a bifunctional aldehyde coupler, we then adsorb a robust NeutrAvidin layer that can be used to immobilize any biotinylated biomolecule and has excellent nonfouling properties. We will describe the surface chemistry and compare our approach with silane-based methods as analyzed by XPS using fluorinated benzaldehyde. We will also show that this chemistry can be successfully applied to GaN surfaces, and used for both immunoassays and DNA hybridization assays in a range of sample matrices.

10:40am **BI-WeM9 Characterization of Surface-Immobilized Layers of Intact Liposomes by Atomic Force Microscopy Force Measurements and Quartz Crystal Microbalance**, ***H. Brochu,*** *P. Vermette,* Université de Sherbrooke, Canada

The scientific literature is abundant on the development, characterization and validation of liposome suspensions, particularly in the biomedical fields. However, much less is known on the surface immobilization of layers of intact liposomes, which can find applications in several fields including drug-partitioning chromatography, cell structure mimicking, and localised drug delivery. In fact, surface-bound liposomes have been validated as localized drug delivery systems in some applications. Although some papers are available on the characterization of surface-immobilized layers of intact liposomes, many physicochemical properties of these complex thin layers still need to be elucidated. Briefly, these surfaces are made

using radiofrequency glow discharge deposition to coat a surface with a thin polymeric film bearing amine groups with the subsequent covalent attachment, using carbodiimide chemistry, of NHS-poly(ethylene glycol)-biotin (NHS-PEG-biotin) low-fouling polymer prepared under cloud point conditions. Next, NeutrAvidin (NA) molecules are docked on the PEG layers bearing biotin followed by the attachment of biotinylated liposomes, which anchor to the NA. X-ray photoelectron spectroscopy is used to follow-up the chemical surface modification steps. This talk will present surface characterization of layers of intact liposomes by AFM force measurements and QCM analysis with dissipation monitoring with the aim to develop a model of the mechanical properties of these thin layers. With AFM force measurements, estimation of the Young modulus of these well hydrated layers is obtained using the Hertz model. QCM measurements allowing dissipation monitoring provide some information on the mechanical properties of these surface-bound liposomes. QCM is also used to study the dynamics of protein adsorption on these surface-bound liposomes. Also, kinetics of the sequential release of more than one molecules from these layers of intact liposomes is investigated.

11:20am **BI-WeM11 The Clinical Performance of Biomimetic Interfaces: the Long and Winding Road***, **K.E. Healy**,* University of California at Berkeley **INVITED**

For nearly two decades, biomimetic or bio-inspired interfaces have been designed to control both the adsorption of macromolecules and cell fate in the peri-implant region. Although successful performance of biomimetic interfaces has been frequent in the biotechnology and biosensor arena, translation of in vitro efforts into the clinical domain have largely failed. Lack of success can be attributed to complex in vivo microenvironments in the peri-implant region that encompass hypoxia, degradative molecules, and fibrin clot formation that can mask biomimetic surface engineering strategies. These microenvironments are not recapitulated using in vitro models, which leads to the poor efficiency of translational research. This lecture will emphasize the universal nature of biomimetic modification strategies and characterization modalities in the context of surface-mediated photoinitiated polymerization to create nanoscale polymer coatings that control the presentation of ligands for cell adhesion and subsequently cell fate determination. The limitations of this biointerface design approach for in vivo applications and current strategies for clinical success will be addressed.

12:00pm **BI-WeM13 Bioarrays with 10,000 Functionalized Spots per Square cm***, **M.R. Linford**, M.C. Aplund, F. Zhang, G. Saini,* Brigham Young University

We describe here a method for very rapidly patterning surfaces to make bioarrays. In this method, a silicon surface is first coated with a monolayer to make it hydrophobic. A microlens array is then positioned over the surface and a single shot (4 ns) from a YAG laser is directed through it. The microlens array focuses the laser pulse into a myriad of tiny spots on the surface that cause removal of the monolayer, leaving ca. 20 micrometer hydrophilic spots. The density of these hydrophilic spots is 10,000 per square cm, i.e., the center-to-center distance between the spots is 100 micrometers. It is then shown by fluorescence and time-of-flight secondary ion mass spectrometry (ToF-SIMS) that polylysine will preferentially adsorb onto the hydrophilic spots. Bioconjugate chemistry from this polylysine base, e.g., the use of phenylenediisothiocyanate as a crosslinker, then allows amine-terminated oligonucleotides and proteins to be attached at the functionalized spots. This bioconjugate chemistry is confirmed on larger, planar surfaces by wetting, optical ellipsometry, X-ray photoelectron spectroscopy, ToF-SIMS, and fluorescence.

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