Biomaterial Interfaces
Room 307 - Session BI+SS-WeA

Biomolecular Surface Science and Microfluidics
Moderator: M. Grunze, Universität Heidelberg, Germany

2:00pm **BI+SS-WeA1 Soft X-ray SpectroMicroscopy of Bio-interfaces**, ***A.P. Hitchcock***, *C. Morin, T. Araki, J.L. Brash, R. Cornelius,* McMaster University, Canada; *S.G. Urquhart, U.D. Lanke,* University of Saskatchewan, Canada; *N. Samuel, D.G. Castner,* University of Washington **INVITED**

We are using scanning transmission x-ray microscopy (STXM) and X-ray photoemission electron microscopy (X-PEEM) to study the adsorption of biological and bio-active species on naturally and artificially patterned polymer and molecular substrates. In one area, the thrust is to investigate fundamental issues of protein - polymer interactions relevant to blood compatibility. In a second area, we are providing feedback for the development of patterned bio-active surfaces. Both synchrotron-based microscopies provide chemical speciation by near edge X-ray absorption spectroscopy (NEXAFS). STXM provides quantitative chemical mapping at a spatial resolution of 50 nm, with the possibility of detecting proteins on polymer thin films with monolayer sensitivity in the presence of an aqueous overlayer of the protein solution.@footnote 1@ X-PEEM has greater surface sensitivity than STXM but requires placing the sample in vacuum. Our X-PEEM studies of fibrinogen adsorption on a model polymer substrate, phase segregated polystyrene-poly(methylmethacrlyate) (PS/PMMA),@footnote 2@ indicate clear preference for adsorption on the PS domains when adsorption is performed from a phosphate buffer solution, but preference for adsorption at the PS-PMMA interface when using non-buffered solutions. The strengths, limitations, and future potential of soft X-ray microscopy for studies of bio-interfaces will be discussed.@footnote 3@ @FootnoteText@ @footnote 1@ A.P. Hitchcock et al., J. Biomaterials Science, Polymer Ed. 13 (2002) 919.@footnote 2@ C. Morin et al, J. Electron Spectroscopy 121 (2001) 203.@footnote 3@ X-ray microscopy carried out at the Advanced Light Source (supported by DoE under contract DE-AC03-76SF00098) and the Synchrotron Radiation Centre (supported by NSF under award DMR-0084402). Research supported financially by NSERC (Canada) and the Canada Research Chair Program. We thank the PEEM-2 staff (A. Scholl, A. Doran) for assistance in these studies.

2:40pm **BI+SS-WeA3 Interaction of Protein Solutions with Biocompatible Organic Monolayers: An In Situ Neutron Reflectometry Study**, ***R. Dahint***, *D. Schwendel,* University of Heidelberg, Germany; *F. Schreiber,* University of Oxford, UK; *M. Grunze,* University of Heidelberg, Germany

Oligo(ethylene glycol) (OEG) terminated self-assembled monolayers (SAMs) effectively prevent the adsorption of proteins from biological solutions. Yet, efforts are still being made to elucidate the mechanisms of protein resistance on a molecular level. For proteins deposited on the tip of an atomic force microscope (AFM), long range repulsive forces have been observed upon approaching protein resistant methoxy-terminated tri(ethylene glycol) undecanethiolate SAMs (EG3-OMe) on gold.@footnote 1@ However, as proteins adsorbed on the tip may undergo significant structural changes, it is not obvious that the same strength and type of interaction is experienced by freely moving, dissolved molecules. We, therefore, used neutron reflectometry to investigate protein/surface interactions employing biomolecules in their native state and natural environment. Room temperature measurements on protein resistant films of EG3-OMe in contact with bovine serum albumin (BSA) solutions reveal the presence of an extended protein depletion layer with a thickness of about 50 nm between the SAM and the bulk protein solution. The results are compared to the strength and range of repulsive forces measured by AFM. Temperature dependent studies on the EG3-OMe/water interface reveal, that a previously observed, density reduced water phase in the vicinity of the SAM cannot account for the protein resistant properties of the films. @FootnoteText@@footnote 1@ K. Feldman, G. Hähner, N. D. Spencer, P. Harder, M. Grunze, J. Am. Chem. Soc. 1999, 121, 10134.

3:00pm **BI+SS-WeA4 Characterization of Lipid Bilayers on Functionalized Surfaces**, ***T.W. McBee***, *S. Saavedra,* University of Arizona

The utilization of planar supported lipid bilayers (PSLBs) for technological applications is limited by their fragility. They can be destroyed by a variety of conditions, including exposure to air, surfactants, and mechanical stress such as elevated temperatures. One way to overcome this limitation is to introduce polymerizable groups into the tail region of the lipid molecules and stabilize the bilayer structure through polymerization, which results in a very stable film when formed on silica. We have been investigating the characteristics of lipid bilayers, both polymerized and unpolymerized, on a variety of functionalized surfaces, including self-assembled monolayers (SAMs) as well as self-assembled polymer multilayers compared to bilayers formed on silica. This talk will focus on our investigations of PSLBs on Î³-aminopropyl silane (GAPS) monolayers as well as on mixtures of polyaniline/poly(acrylic acid). These types of surfaces are of interest due to their potential for energy transduction and sensing applications.

3:20pm **BI+SS-WeA5 Intact Vesicle Adsorption and Supported Biomembrane Formation from Vesicles in Solution Studied by a Combined SPR and QCM-D Instrument, and AFM**, *E. Reimhult*[1], *F. Höök,* ***B. Kasemo***, Chalmers University of Technology, Sweden

Two biomembrane model systems receiving high interest are unilamellar phospholipid vesicles and supported planar phospholipid bilayers. We have investigated the adsorption kinetics of small unilamellar POPC vesicles with a setup, combining the Quartz Crystal Microbalance with Dissipation technique and Surface Plasmon Resonance techniques in parallel. Using this instrument we have for the first time simultaneously and in real time measured the acoustic (hydrated) and optical (dry) mass for vesicle adsorption on SiO@sub 2@ and oxidized Au. These measurements have significantly extended our understanding of intact vesicle adsorption on surfaces, as a function of surface chemistry,@footnote 1@ temperature,@footnote 1,2@ vesicle size,@footnote 3@ and osmotic stress.@footnote 1@ We have been able to distinguish between the mass response due to adsorbing intact vesicles and that of planar bilayers formed on the surface via vesicle rupture. Furthermore, we have; determined the critical coverage required for vesicle rupture on SiO@sub 2@, obtained a measure of the amount of trapped water and deformation of intact vesicles on the surface, studied the kinetics of the final part of the supported planar bilayer formation process on SiO@sub 2@ and detected loss of lipids on its completion. The latter two are critical for the quality of the formed bilayer, i.e. limiting the number of defects. We also intend to present results from atomic force microscopy studies of vesicle adsorption on heterogenous (patterned) surfaces, with focus on the behavior at phase boundaries, separating planar bilayer forming and intact vesicle adsorbing areas. @FootnoteText@ @footnote 1@ E. Reimhult, F. Höök, and B. Kasemo, Langmuir 19, 1681 (2003)@footnote 2@ E. Reimhult, F. Höök, and B. Kasemo, PRE 66, 051905 (2002).@footnote 3@ E. Reimhult, F. Höök, and B. Kasemo, JCP 117, 7401 (2002).

3:40pm **BI+SS-WeA6 Microfluidic Systems for Applications in Chemistry and Biochemistry**, ***A. Manz***, Imperial College, UK **INVITED**

Fluid handling integrated into microsystems has been in use now for a number of years. Mostly, research has focused on micro pumps, valves, sensor flow cells and electrophoresis.@footnote 1,2,3@ However, the underlying idea of shrinking the whole analytical chemistry or biochemistry lab down to chip size will make it necessary to talk about interfacing these modules properly and efficiently. Recently, we have proposed a concept for a chemical microprocessor.@footnote 4,5@ This concept is related to an earlier attempt to define an analytical chemistry microsystem, micro-TAS (miniaturised total analysis system).@footnote 6@ Scaling laws predict 100x faster mass and heat transport, if a known system is miniaturized by a factor 10 (linear). This is particularly useful in capillary electrophoresis, chromatography and continuous-flow reactors. We have presented several examples in the past few years. I plan to show a few examples of recent chip developments taken from my lab: A horseradish peroxidase assay (400ms incubation), isoelectric focusing of a peptide in continuous flow (100x higher concentration in 300ms focusing time) and an air monitor based on a plasma emission chip. @FootnoteText@ references @footnote 1@Micro Total Analysis Systems 2000, A.van den Berg, W.Olthuis, P.Bergveld, eds., Kluwer Academic Press, ISBN 0-7923-6387-6 (2000). @footnote 2@D.R.Reyes, D.Iossifidis, P.A.Auroux, A.Manz, Anal Chem 74, 2623-2636 (2002) @footnote 3@P.A.Auroux, D.Iossifidis, D.Reyes, A.Manz, Anal Chem 74, 2637-2652 (2002) @footnote 4@A.Manz, H.Becker, Transducers 97, Chicago, June 16-19, 1997, Digest of technical papers (1997) 915-918. @footnote 5@M.C.Mitchell, V.Spikmans, A.Manz, A.J.de Mello, J.Chem.Soc., Perkin Trans.1 2001 (2001) 514-518. @footnote 6@A.Manz, N.Graber, H.M.Widmer, Sens. Actuators B1 (1990) 244-248.

4:20pm **BI+SS-WeA8 Interfacial Engineering for Protein Biochips in Proteomics Applications**, ***H. Lu***, *P. Kernen, P. Wagner,* Zyomyx, Inc.

Proteomics is increasingly dependent on analytical tools that focus on quantification of protein expression, biomolecular-protein interactions, and

[1] **Morton S. Traum Award Finalist**

functional activity. The large numbers of proteins and complexity involved in proteomics applications present tremendous challenges for the development of analytical platforms and specifically interfacial engineering schemes. Zyomyx has developed a novel protein biochip platform that facilitates rapid, precise, highly multiplexed analysis with minimal sample requirements and has integrated several sophisticated interfacial engineering strategies. The biochip architecture consists of a three-dimensional array structure designed to provide consistent feature size and defined placement, while eliminating spot-to-spot cross contamination. We will focus on details of biochip development with an emphasis on organic layer compositions for optimal packing density, molecular orientation, selective immobilization of capture reagents, and low non-specific protein adsorption. Examples on high-level quantitative protein analysis will focus on Zyomyx human Cytokine Biochip capable of fully multiplexed and quantitative protein analysis based on sandwich-immunoassay configurations.

4:40pm **BI+SS-WeA9 Arrays of DNA-tagged Vesicles Based on Spontaneous Sorting to a DNA-array Template**, ***I. Pfeiffer***, *S. Svedhem, F. Höök,* Chalmers University of Technology, Sweden

We have developed a surface-modification protocol that allows sorting of DNA-tagged vesicles (where DNA is anchored via a cholesterol moiety) to arrays of cDNA-modified Au-spots on a SiO@sub 2@ surface. Biotinylated albumin (biotin-BSA) was chosen to functionalize Au spots surrounded by SiO@sub 2@, while supported phospholipids bilayers was formed on the surrounding SiO@sub 2@, thus representing an inert background. This allowed subsequent coupling of biotinylated DNA strands via neutravidin bound to biotin-BSA only on Au spots. Eventually, a cDNA array created in this way was proven compatible with specific immobilization of differently DNA-tagged vesicles utilizing complementary DNA hybridization. The surface functionalization protocol was established using the quartz crystal microbalance technique with dissipation monitoring (QCM-D), allowing quantification of the different immobilization steps, while fluorescence microscopy was used to analyze the vesicle sorting. The compatibility of the vesicles to act as carriers for proteins was proven using scFv-antibodies, anchored to the DNA-tagged vesicles via nitrilotriacetic acid (NTA)-functionalized lipids. These results thus open up the prospect to use surface directed sorting of functionalized vesicles for construction of protein arrays, avoiding complicating and/or destructive microfluidics or microdispensing protocols. The work also includes a number of alternative approaches towards the creation of DNA arrays being compatible with the above described principle for spontaneous vesicle sorting, including improvements in the strength of the cholesterol-DNA based coupling and the dimension of the arrays, which has the potential to be down-scaled to the length of the DNA probes and the size of the vesicles, typically being less than 100 nm.

5:00pm **BI+SS-WeA10 Immobilization of Oriented Protein Molecules on High-density Poly(ethylene glycol) Coated Si(111)**, ***T. Cha,*** University of Minnesota*; A. Guo,* MicroSurfaces, Inc.*; X.-Y. Zhu,* University of Minnesota

The success of DNA microarray technology has motivated the development of similar tools for proteins. One of the key challenges in this chip-based assay is how the liquid-solid interface is engineered to minimize nonspecific adsorption, to control protein conformation and orientation, and to present high specificity for protein attachment. We demonstrate the synthesis of high density poly(ethylene glycol)-coated Si(111) and its application as an excellent substrate for protein microarray technology. The surface is obtained from the reaction of a multi(8)-armed PEG (mPEG) molecule with a chlorine terminated Si(111) surface to give a PEG film with thickness of 5.2 nm. Four out of the eight arms on each immobilized PEG molecule are accessible for linking to the chelating iminodiacetic acid (IDA) group which binds Cu2+ ions (2.7x10^13/cm2). The resulting Cu2+-IDA-mPEG-Si(111) surface is shown to specifically bind 6x-histidine-tagged protein molecules, including green fluorescent protein and sulfotransferase, without the need of pre-purification. In the case of 6xHis-GFP, this immobilization strategy can lead to a closely packed monolayer of protein molecules. Background tests show that the surface retains its inertness towards non-specific protein adsorption in the absence of either a poly-His tag on the protein molecule or metal ions on the surface. Both the inertness of the chemical surrounding and the controlled orientation contribute to an ideal environment for the immobilized protein molecule to retain its native conformation and reactivity. Enzyme activity for surface immobilized sulfotransferase are measured and compared to solution phase values. Such a kinetic experiment essentially extends a 2-dimensional array to the third dimension-time.

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