Friday Morning, October 29, 1999

Biomaterial Interfaces Group
Room 613/614 - Session BI-FrM

Interface, Properties, and Modification

Moderator: B.D. Ratner, University of Washington

8:20am **BI-FrM1 Topographical Polymorphism of a Phospholipid Monolayer**, ***W.R. Schief***, *L.A. Touryan,* University of Washington; *S.B. Hall,* Oregon Health Sciences University; *V. Vogel,* University of Washington

Light scattering microscopy reveals previously undetected topographical complexity in lipid monolayers at the air/water interface. At a surface pressure (@pi@) of @pi@ = 13 mN/m at room temperature, following completion of the liquid-expanded (LE) -> liqu id-condensed (LC) transition, the LC phase of Dipalmitoylphosphatidylcholine (DPPC) develops corrugations within a region covering half the monolayer and surrounding flat, chiral-shaped domains. The scattered intensities of the domains and the surrounding region are analyzed in light of capillary wave theory. With compression over @pi@ = 20 mN/m, the corrugated region becomes decorated with nanoparticles through a reversible budding process. Beyond a threshold of @pi@ = 60 mN/m, the budding accelerates. A tomic force microscopy (AFM) on samples transferred to mica confirms the presence of multibilayer discs of diameter 15 - 150 nm. These findings provide new information on potential surface mechanisms of respiration, since a monolayer enriched in DPPC is widely thought to coat the lung.

8:40am **BI-FrM2 Plasma Co-polymer Surfaces for the Controlled Adsorption of Common Proteins**, ***J.D. Whittle***, *R.D. Short, C.W.I. Douglas,* University of Sheffield, UK; *J. Davies,* Johnson and Johnson Orthoclinical Diagnostics, UK

The topic of protein adsorption is of key interest in biomaterials science, since it is generally believed that subsequent surface reactions are guided by the composition of adsorbed proteins. This is especially pertinent in immunodiagnostics, where nonspecific and poorly characterised protein binding may lead to false positives and poor signal-noise ratios. We are interested in controlling the passive adsorption of several common proteins from single solutions through the molecular engineering of surfaces by means of plasma. We utilise continuous wave rf plasma copolymerisation to allow us to synthesise ultra-thin plasma polymer (PP) films of controllable surface chemistry, from various starting 'monomers'. The plasma polymers are analysed by XPS to allow us to estimate the proportion of different functional groups present in the deposited surface. In this experiment we examine surfaces deposited from plasmas of allyl alcohol and acrylic acid. A range of surface functionalities was produced by copolymerising the functional monomer with 1,7-octadiene. Protein adsorption was estimated by an Enzyme Immunoassay (EIA) after exposing the surfaces to single solutions of human albumin, fibrinogen, vitronectin and IgG overnight at a temperature of 37°C and a pH of 7.0. Results show that the amount of protein adsorbed depends not only upon the protein being investigated, but also the characteristics of the polymer surface, with a clear correlation between functional group concentration and the adsorption of fibrinogen, vitronectin and IgG. The adsorption of albumin is not affected by the funtionality of the surface, however this may reflect a limitation in the technique. SPR measurements suggest that even at low concentrations albumin can form a multilayer on these plasma deposited surfaces, which would lead to poor sensitivity of the assay.

9:00am **BI-FrM3 Reversible Immobilization of a Thermally-Responsive Fusion Protein on a Hydrophobic Surface**, ***W. Frey***, *N.A. Hattangadi, D.E. Meyer, A. Chilkoti,* Duke University

Elastin-like polypeptides (ELPs), which are composed of repeats of the Val-Pro-Gly-X-Gly (X is a variable residue) pentapeptide, undergo a phase transition as a function of temperature. ELPs are soluble in water below their transition temperature, and are insoluble in water and aggregate when the temperature is raised above the transition temperature. We have synthesized a thioredoxin-ELP fusion protein (Trx-ELP), and shown by ellipsometry, surface plasmon resonance spectroscopy, and atomic force microscopy that below its transition temperature, soluble Trx-ELP does not interact with a hydrophobic surface. However, above the transition temperature, insoluble Trx-ELP forms an adsorbed monolayer on a hydrophobic surface, in which Trx is oriented towards the bulk. Adsorbed Trx-ELP binds an anti-thioredoxin monoclonal antibody with high affinity from solution. The Trx-ELP/antibody complex can be resolubilized from the surface by reducing the solution temperature below the transition temperature. The adsorption transition is driven exclusively by the hydrophobic surface, because no adsorption is observed on a hydrophilic surface, and previously aggregated Trx-ELP in solution does not adsorb onto a hydrophobic surface. The adsorbed Trx-ELP complex shows micelle-like organization, with a mean diameter of approximately 100 nm. Current studies on reversible self-organization of ELP fusion proteins onto micro and nano-patterned surfaces, based on an easily controllable solution parameter, suggest their application in biosensor development and modulation of cell-substrate interactions.

9:20am **BI-FrM4 The Fibronectin Type III Domain: A Scaffold for a Molecular Recognition Switch**, ***A. Krammer***, University of Washington; *H. Lu, B. Isralewitz, K. Schulten,* University of Illinois, Urbana-Champaign; *V. Vogel,* University of Washington

The forced unfolding of fibronectin's tenth type III module (FnIII10) was simulated by steered molecular dynamics (SMD) indicating that mechanical tension applied to the module's termini renders its RGD loop inaccessible to cell surface integrins. FnIII10 possesses a beta-sandwich motif consisting of seven beta-strands (A-G) that are arranged in two antiparallel sheets with the RGD peptide sequence located at the apex of the FG loop. Computer simulations now reveal that the b-strand G separates from the module at an early stage of unfolding while the remaining fold experiences only small structural perturbations. Consequently, the RGD peptide is pulled closer to the module's surface as the FG loop unravels. A molecular scale picture of the forced unfolding pathway will be discussed as well as its implications for the understanding of cell-surface interactions.

9:40am **BI-FrM5 Utilizing Direct Communication between Enzymes and Conducting Polymers in Glucose Sensors**, ***A. Kros***, University of Nijmegen, The Netherlands; *S.W.F.M. van Hövell,* TNO Nutrition and Food Research Institute, The Netherlands; *D.M. Vriezema, R.J.M. Nolte,* University of Nijmegen, The Netherlands

Biosensors are currently of great interest because of the potential to measure a variety of substrates like glucose and lactate.@footnote 1@ Glucose is by far the most studied analyte in this field of research, primarely due to its importance in human metabolic processes. Here we report the development of a glucose sensor, which will be utilized to measure in vivo glucose levels in the near future. The working mechanism of the sensor is based upon direct electron communication between the enzyme glucose oxidase and a conducting polymer.@footnote 2@ In this new glucose sensor, ethylenedioxythiophene is polymerized chemically inside the pores of a cyclopore track-etch membrane using iron(III)chloride as a catalyst. In this way, a thin layer of conducting polymer (polyethylenedioxythiophene, pedot) is deposited in the interior of the pores. The latter layer is subsequently covered with the redox enzyme glucose oxidase by means of physical adsorption and electrostatic interactions between the positively charged pedot and the negatively charged enzyme. The resulting sensor is able to detect glucose in the clinical relevant concentration range via amperometric methods. The influence of electrostatic interactions and the use of electronic mediators on the sensor performance will be discussed. @FootnoteText@ @footnote 1@ A.E.G. Cass, A practical approach, Oxford University Press, New York, 1994. @footnote 2@ C.G.J. Koopal, B. de Ruiter, R.J.M. Nolte, J. Chem. Soc. Chem. Commun., 1991, 1691.

10:00am **BI-FrM6 Cap-shaped Gold Nano Particles for Optical Biosensing**, ***M. Himmelhaus***, *H. Takei,* Hitachi, Ltd., Japan

Gold nano particles can be utilized for optical detection of biomolecules.@footnote 1, 2@ The approach presented here is well suited for the development of miniaturized, inexpensive biosensors for two reasons. First, the preparation of the sensing surface is easy to control and highly reproducible. Second, the unique optical properties of cap-shaped gold particles, such as a pronounced reflectivity minimum in the visible region of OD. 2.4 with a bandwidth of 100 nm, lead to a highly sensitive though simple optical read-out quantity. For preparation, a gold layer of 20 nm is first evaporated on a polystyrene (PS) substrate. Then, the gold layer is exposed to an aqueous PS nano sphere suspension containing a small amount of carbodiimide. Addition of the last chemical to the commercially available monodisperse PS sphere suspension leads to formation of a dense monolayer of randomly positioned PS nano spheres on the gold thin film. After one hour of incubation, superfluous PS spheres are simply washed off with deionized water. Finally, a gold layer of 20 nm thickness is evaporated onto the PS sphere monolayer leading to formation of cap-shaped gold particles. The resulting surface exhibits a pronounced extinction peak upon reflection of visible light. The shift of this reflectivity minimum due to changes in the refractive index of the immediate environment can be monitored with simplest optical methods and therefore is well adapted to

miniaturization. We will show that a straightforward fiber-optical setup is sensitive enough to detect in-situ monolayer formation of alkanethiolates. Further, the capability of sensing biomolecular adsorption will be demonstrated utilizing the biotin/avidin functional pair as a model system. @FootnoteText@ @footnote 1@R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger and C. A. Mirkin, Science 277 (1997) 1078-81 @footnote 2@F. Meriaudeau, T. R. Downey, A. Passian, A. Wig and T. L. Ferrell, Appl. Opt. 37 (1998) 8030-7.

10:20am **BI-FrM7 Properties of Thiol Monolayers in Contact with Liquids: An In Situ Investigation by IR-vis Sum Frequency Spectroscopy***, **M. Zolk**, F. Eisert, M. Buck, M. Grunze,* University of Heidelberg, Germany

In the vast majority of cases the analysis of the structure and properties of self-assembled monolayers (SAM) takes place ex situ, e.g. in air or even under vacuum. In contrast, the application in fields relevant to biotechnology requires a detailed understanding of their properties in contact with liquids, in particular aqueous solutions. This raises the question of the relevance of ex situ investigations to conditions relevant for applications. We report experiments on SAMs of thiols on gold and metal substrates. The behavior of pure and end group modified thiols in contact with different liquids is studied. In particular, we focus on SAMs which consist of a methoxy terminated ethylene glycol (EG) unit attached to an alkane thiol. Whereas SAMs of pure alkane thiols are affected relatively little by solvents, EG-SAMs which are important for the preparation of protein resistant surfaces, exhibit a drastic dependence on the environment. All solvents examined penetrate the layer and induce significant conformational changes. Compared to air the signal from the methoxy end group is strongly reduced in contact with solvents. This indicates a transition from a well-ordered to an amorphous state. Analogously, the methylene vibrations gain intensity and thus indicate an increase of gauche conformations. Depending on the solvent the degree of penetration is different. A polar solvent such as water mainly interacts with the EG part of the layer whereas non-polar solvents interact as well with the hydrocarbon chain and thus penetrate deeper into the SAM. The experiments clearly demonstrate the need to investigate SAMs under conditions relevant for applications.

10:40am **BI-FrM8 Biofilm - Titanium Chemistry of Adhesion Using X-ray Photoelectron Spectroscopy***, **R.A. Brizzolara**,* NSWC - Carderock Division

Virtually all surfaces immersed in water for any period of time are colonized by microorganisms. These organisms adhere to the surface by producing extracellular polymers, predominantly polysaccharides. Biofilm formation and resulting biofouling cause serious problems for heat transfer equipment due to inhibited water flow and degradation of the heat transfer coefficient. Conventional coatings cannot be applied to heat transfer materials due to degradation of the heat transfer coefficient. Titanium, often the material of choice for heat transfer applications because of its corrosion resistance, is very prone to biofouling. Materials and/or surface modification strategies to decrease the strength of adhesion or the rate of biofilm formation would be of great value. As a first step in developing such a strategy, the interfacial chemistry between biofilm components and titanium is being investigated. This paper reports on the use of x-ray photoelectron spectroscopy to examine the interfacial chemistry between alginic acid and n-acetyl glucosamine and titanium. XPS is used to quantify the adsorbate bound to the surface under various conditions (including pH and salt content of the water), and to evaluate the adsorbate-surface bonding mechanism. Information regarding the biofilm-surface chemical interaction will be useful in developing better fouling resistant surfaces. The NSWC Carderock Division In-House Laboratory Independent Research Program supported this work.

11:00am **BI-FrM9 Planar Polymerized Phospholipid Bilayers as Biocompatible Substrates***, **J.C. Conboy**, S. Liu, D.F. O'Brien, S.S. Saavedra,* University of Arizona

There is considerable interest in finding a surface that is resistant toward non-specific protein adsorption and is chemically and mechanically stable. Hydrophilic surfaces, such as those of a zwitterionic phospholipid bilayer, are inherently biocompatible with intrinsically low nonspecific protein interactions. However, planar supported lipid bilayers are only weakly associated, making their stability less then desirable from an applications standpoint. Toward the goal of producing a stable and intrinsically biocompatible substrate, we endeavored to produce planar polymerized analogs of phospholipid bilayers. A photosensitization method was used to polymerize the lipid bilayers in aqueous media. The rate of polymerization and subsequent structural changes in the lipid film were examined by in-situ Raman spectroscopy. The stability of the lipid films was determined before and after in-situ polymerization by a number of methods. Their application as substrates for optical biosensors will also be discussed.

11:20am **BI-FrM10 Nanoscale Patterning of Gold for Attachment of Supported Lipid Bilayers***, **A.T.A. Jenkins**,* Max-Planck Institut für Polymerforschung, Germany

Attaching lipid bilayers to solid substrates in such a way that they exhibit properties analagous to cell membranes found in Nature is becoming of increasing interest. Such systems have the potential to be used as biosensors and for fundamental studies of cell membranes. In this paper we present a novel method of attaching such lipid bilayers to gold substrates using microcontact printing to produce a patterned surface of sub-micron size patterns onto which a lipid layer is added. Microcontact printing has been used to form patterns of lipophilic Self-assembled monolayers (SAMs) on gold with dimensions of 500 nm or less. These patterns consist of a regular array of hydrophilic and hydrophobic patches. Onto these patterned SAMs, lipid bilayers have been formed over the hydrophilic patches by lipid vesicle rupture and self-assembly. Investigation of lipid bilayers on these small nanometer scale patterns compared with larger micrometer scale patterns of lipopilic SAMs, by both Impedance Spectroscopy and Surface Plasmon Spectroscopy have suggested that vesicle adsorption followed by rupture at hydrophilic-hydrophobic SAM interfaces may be a crucial part of the mechanism of bilayer formation on such patterned SAMs. Finally, ion-selective peptides and proteins including Valinomycin and Gramicidin have been inserted into the bilayer patches, and the expected ion-selectivity observed experimentally.

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