## Biomaterial Interfaces
## Room: Hall D - Session BI-TuP

## Biomaterials Interfaces Poster Session with Focus on Cells and Proteins at Interfaces

**BI-TuP1 Human Serum Albumin Adsorption to Expanded Polytetrafluoroethylene**, ***E.S. Leibner***, *N. Barnthip, W. Chen, C.R. Baumrucker, J.V. Badding,* The Pennsylvania State University, *M.V. Pishko,* Texas A&M University, *E.A. Vogler*, The Pennsylvania State University

Analytical protocol greatly influences measurement of human-serum albumin (HSA) adsorption to commercial expanded polytetrafluororethylene (ePTFE) exhibiting superhydrophobic wetting properties (water contact angles in excess of 150°). Degassing of buffer solutions and evacuation of ePTFE adsorbent to remove trapped air immediately prior to contact with protein solutions are shown to be essential steps that assure reproducible measurement of HSA adsorption. Results obtained with ePTFE as a superhydrophobic test material suggest that vacuum degassing should be generally applied in the measurement of protein adsorption to any surface exhibiting superhydrophobicity. Solution depletion quantified using radiometry (I-125 labeled HSA) or electrophoresis (unlabeled HSA) yield different measures of HSA adsorption, with nearly seven-fold higher surface concentrations of unlabeled HSA measured by the electrophoresis method. This outcome is attributed to the influence of the radiolabel on HSA hydrophilicity which decreases radiolabeled-HSA affinity for a hydrophobic adsorbent in comparison to unlabeled HSA. These results indicate that radiometry underestimates the actual amount of protein adsorbed to a particular material. Removal of radiolabeled HSA adsorbed to ePTFE by 3X serial buffer rinses also shows that the remaining "bound fraction" was about seven-fold lower than the amount measured by radiometric depletion. This observation implies that measurement of protein bound after surface rinsing significantly underestimates the actual amount of protein concentrated by adsorption into the surface region of a protein-contacting material.

**BI-TuP2 The Role of Surface Chemistry in poly-L-lysine Mediated Alginate Adsorption**, ***K.T. Queeney***, *J.E. Brown, E.K. Davis, C. Franqui*, Smith College

Adsorption of polysaccharides to solid substrates, an essential component of biofilm formation, is generally protein-mediated. Lysine-rich proteins, in particular, are known to have significant adhesive properties in these systems. This work uses poly-L-lysine (PLL) as a model protein to explore the role of the underlying substrate in protein-mediated adsorption of alginate, a negatively-charged polysaccharide. We have prepared films of varying PLL coverage on two types of surfaces: hydrophilic, negatively-charged silica and a hydrophobic, electrically neutral alkylsilane monolayer. PLL coverage on the hydrophilic surface is controlled by varying the ionic strength of the adsorption solution, while PLL coverage on the hydrophobic surface is controlled by varying the molecular weight of the PLL. By a combination of techniques--primarily ellipsometry, dynamic contact angle goniometry and atomic force microscopy--we demonstrate that not only the film morphology of the PLL but also its effect on subsequent alginate adsorption is highly sensitive to the surface chemistry of the underlying substrate. For example, PLL films on silica are more discontinuous at low and high ionic strength, and alginate adsorption is highly influenced by this discontinuity, which allows for repulsive electrostatic interactions between the surface and the alginate. In contrast, while there is an increase in PLL film discontinuity as a function of increasing PLL molecular weight, alginate adsorption appears largely insensitive to these changes in PLL film morphology. Through quantitative analysis of the advancing contact angles on both these (chemically heterogeneous) surfaces we demonstrate unequivocally that the PLL/solution interface is highly dependent on both substrate surface chemistry and PLL adsorption conditions.

**BI-TuP3 Time of Flight Secondary Ion Mass Spectrometry Characterization of the Surface Polysaccharides of Group B Streptococcus**, *T. Nguyen,* Oregon State University, ***J.R. Hull***, *D.G. Castner*, University of Washington NESAC/BIO

Group B Streptococcus (GBS) is a leading cause of sepsis and meningitis in neonates and immunocompromised adults in western countries. The surface of GBS is well characterized by standard microbiological techniques and therefore makes a good test system for analyzing bacteria using Time of Flight Secondary Ion Mass Spectrometry. GBS is layered with a capsule composed of five distinct polysaccharides containing glucose, galactose, N-acetylneuraminic acid, rhamnose, and N-acetylglucosamine. The capsule makes up 10 to 30% of the dry weight of the microorganism and is only present on the surface. In this work, the five monosaccharides, pure capsule from type III GBS, and UV killed GBS strain COH1 and acapsular GBS strain COH1-13 were investigated. It was observed that the pure monosaccharide fragmentation patterns followed $C_xH_{2x}O_x^+$, and that the largest fragment observed of the monosaccharides was with the loss of $H_2O$, $OH^-$ or $H_3O^+$. The presence of sodium, magnesium, and calcium in the purified samples made direct comparison with the pure monosaccharides difficult due to cationization effects. Spectra from clusters of GBS and single organisms were acquired using the high mass resolution imaging mode and constructing a spectrum from a region of interest. The biggest differences between COH1 and COH13 were seen in the high mass region of the spectra.

**BI-TuP4 Hydrogels with KGF**, ***J.A. Appawu***, *J.A. Gardella*, University at Buffalo: The State University of New York

XPS and TOF-SIMS are techniques that can be used to quantify the uptake and release of proteins from polymers. Hydrogels are cross-linked hydrophilic polymers used in localized delivery of growth factors through pores defined by type and density of cross-links. Therefore uptake and release are diffusion controlled. Keratinocyte growth factor 1(KGF-1) is known to promote re-epithelialization after skin injury and stimulate the proliferation of skin cells. Inadequate amounts of growth factors can result in inefficient healing. In this study, (hydroxyethyl methacrylate ) (HEMA) hydrogels 3% and 6% were prepared with varying cross-link densities to answer three key questions:1) What is the optimal concentration of KGF-1 for cell adhesion, 2) Are HEMA hydrogels with specific structural properties viable for cell proliferation, and 3) What is the amount and time course of released KGF-1 present on the surface? Hydrogels were cut into equal areas to determine the amount of KGF-1 protein taken into and released from the hydrogels by fluorescence spectroscopy. XPS was used to confirm the quantity of KGF-1 on the surface and bulk by varying the take-off angle (TOA). The nitrogen atomic concentration was used to track the protein since nitrogen is present only in KGF-1. The amount of KGF-1 was higher in the 3% hydrogel due to a swelling ratio of 58.5% compared to 40.5% for the 6% hydrogel. Cell adhesion experiments have shown that 80-90% of HaCaT cells successfully adhere to the surface of the KGF-1 imbibed hydrogels and fluorescence microscopy proved that the cells were alive. TOF-SIMS has been used in depth profiling to confirm the XPS results.

Mahoney C. M., Yu J., Fahey A., and Gardella J. A. Jr. SIMS Depth Profiling of polymer blends with protein based drugs. Applied Surface Science (2006) 6690-6614. Pierce G.F. et. al. Stimulation of All Epithelial Elements during Skin Regeneration by Keratinocyte Growth Factor. J. Exp. Med. (1994) 179, 831-840. Tsuboi R. et al. Keratinocyte Growth Factor (FGF-7) Stimulates Migration and Plasminogen Activator Activity of Normal Human Keratinocytes. J. Invest. Dermatol. (1993) 101, 49-53. Greenhalgh D. G. The role of growth factors in wound healing. J. Trauma. (1996) 41, 159. Mahoney C. M., Yu J., and Gardella J. A. Jr. Depth Profiling of Poly(L-lactic Acid)/TriBlock Copolymer Blends with Time-of-Flight Secondary Ion Mass Spectrometry. Anal. Chem. 2005 77, 3570-3578.

**BI-TuP5 Patterning of pOEGMA Polymer Brushes Using Photolithography and Photomasking for Applications in Protein and Cellular Adhesion Research**, *M.S. Johannes, A. Garcia,* ***A. Hucknall***, *A.J. Simnick, R.L. Clark, S. Zauscher, A. Chilkoti*, Duke University

We report the use of bulk microfabrication processes to pattern non-fouling, surface-initiated poly-oligoethylene glycol methacrylate (pOEGMA) polymer brushes. Previous studies have demonstrated the non-fouling capability of pOEGMA surfaces through protein absorption and cell adhesion studies. Here, we demonstrate that the polymer brushes on a glass substrate can be easily patterned using two processes. In the first one, standard photolithography is used to pattern both negative and positive photoresist spin-coated on the pOEGMA layers. The patterned photoresist layers act as masks for the removal of exposed underlying pOEGMA layers by UV-O3 exposure and oxygen plasma ashing. Upon photoresist stripping, patterns with a resolution down to ~400 nm are achieved. In the second approach, TEM grids are directly placed on the pOEGMA layer to block the brushes from direct exposure to various oxidative species offering great simplicity, high-throughput, and low-cost. Successful patterning of non-fouling surfaces at both the micron and nanometer scale will have importance in developing patterned cell monolayers and studying linear motor protein locomotion.

**BI-TuP6 Novel Patterned Protein Assay To Measure Differential Extracellular Matrix Protein Affinities for Cellular Attachment and Axonal Outgrowth**, ***W.M. Theilacker***, *A.L. Styer, H.P. Bui,* University of Delaware, *D.E. Willis, J.L. Twiss,* Alfred I. DuPont Hospital for Children, *T.P. Beebe, Jr.*, University of Delaware

Cellular preference for extracellular matrix (ECM) proteins was assayed on patterned surfaces presenting two ECM proteins that compete for cell attachment and proliferation. Microcontact printing techniques were used to modify silicon substrates with alternating 40-um-wide stripes of the ECM proteins fibronectin and laminin. The spatial distribution of both proteins on the patterned surfaces was measured by epi-fluorescence and Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS). Attachment and outgrowth of dorsal root ganglia (DRG) neurons and neuron-like pheochromocytoma (PC12) cells on striped substrates were analyzed up to 4 days. At each time point, three patterned samples were fixed and immunostained prior to fluorescence imaging. Images were analyzed for the number of cells attached to each protein region and the number and length of neurite extensions. Results indicate for PC12 cells, an approximately equal number of cells on fibronectin and laminin stripes after 24 hours in cell culture. However, from 48 hours to 96 hours, the number of cells on laminin versus fibronectin continually increased. By 96 hours, 80 percent of the PC12 cells were attached to laminin versus fibronectin. Preliminary results for DRG neurons suggest a similar trend, in addition to the influence of Schwann cells, which are known to influence DRG neurite outgrowth.

**BI-TuP7 XPS Analysis of Protein Adsorption on Bioprocessing Materials**, ***R.A. Zangmeister***, National Institute of Standards and Technology

Protein therapeutics, a fast-growing category of biotech drugs, is adversely affected by protein aggregation. Aggregation can decrease the safety and efficacy of the therapeutic biologic protein product. Currently, the cause of protein aggregation is not clearly understood. The adsorption of protein therapeutics to bioprocessing materials used in production, storage and delivery may create nucleation sites for further protein aggregation. X-ray photoelectron spectroscopy was used to analyze protein adsorption on bioprocessing materials to screen for those with increased probability of contributing to aggregation of the protein therapeutic. The standard overlayer model and protein labeling were used to estimate the thickness of adsorbed protein layers.

**BI-TuP8 Highly Sensitive Probe of Crystalline Sub-Micron Sized Domain Structures in Amorphous Material**, ***H.B. de Aguiar***, *S. Roke*, Max-Planck-Instiut fuer Metallforschung, Germany

Mixtures of solid compounds or phases occur in a wide variety of chemical processes. Nucleation, crystallization and separation of phases are generally integrative steps in the production of polymer compounds, pharmaceuticals and in many other chemical processes. Many techniques used for analyzing mixtures of medium and ingredients in-situ rely on the interaction of photons with the mixture.[1] Such a situation occurs in solid/solid dispersions, where two different phases coexist, as in the case of most polymers which are known to consist of crystalline domains embedded in an amorphous phase. For instance, Wide-Angle X-Ray Diffraction (WAXS) and Small-Angle X-Ray Scattering (SAXS) are used together to obtain crystallite structure, size and fraction, being limited only by the level of background signal relative to the signal itself. We developed a new technique to study crystalline domains dispersed in an amorphous phase through nonlinear optical Vibrational Sum-Frequency Generation (VSFG) Scattering.[2] VSFG is a second-order nonlinear optical process, which is forbidden in centrosymmetric media (e.g. liquids, amorphous solids). We show the higher sensitivity of VSFG scattering to microspheres (MS) consisted of crystalline and amorphous phase. We studied the scattering pattern of some chiral polymers, namely Poly(Lactic Acid) (PLA). MS made of P(DL)LA polymer (polymer chains consisting of a random distribution of enantiometers) were probed by X-Ray Diffraction (XRD) and Differential Scanning Calorimetry (DSC) as well. XRD and DSC showed no crystallinity of the PDLLA MS, in agreement with previously published studies. However, VSFG spectra and scattering patterns demonstrate that there is still a finite number of crystallites with maximum extension of 250 nm (radii). This indicates VSFG has an increase in sensitivity that is roughly 2 orders of magnitude more than XRD.[3] This opens up new possibilities in the description of early stages of nucleation and growth phenomena and possible new phases.

[1]S Roke et al. Vibrational Sum Frequency Scattering from a Submicron Suspension. Phys. Rev. Lett. 91, 2003, 258302.

[2]AGF de Beer et al. Molecular and microscopic properties of buried microstructures. Submitted.

[3]HB de Aguiar and S Roke. Sum-Frequency Generation Scattering: a higher sensitivity probe for crystalline properties. In preparation.

**BI-TuP9 Effect of Film Fabrication Method on the Protein Fouling Resistance of Mixed Oligo(ethylene glycol) Films**, ***J.A. Ruemmele***, *M.S. Golden, R.M. Georgiadis*, Boston University

A major concern in the design of biosensors for studying protein interactions is protein fouling, or non-specific binding, which masks the signal from the binding event of interest. Mixed oligo(ethylene glycol) (OEG) films have become a standard surface coating used to avoid fouling of gold sensor surfaces. Such films comprise a probe coupling OEG (A) and a diluent of protein resistant OEG (B). Two methods exist for the fabrication of such surfaces: co-deposition of A and B, and sequential deposition, where A is inserted into a pre-existing monolayer of B. Though films fabricated by sequential deposition display better fouling resistance, the source of this resistance is unclear. It has been hypothesized that the improved fouling resistance is due to an absence of islanding in the film, however it could simply result from an extremely low A content. In order to identify the affect of the fabrication method, films prepared by both approaches must contain the same amount of A. Since few techniques exist which can accurately measure low amounts of A attached to a surface, the amount of probe which covalently couples to a film was used to evaluate the film's A content. Using this technique, the fabrication conditions for both methods were adjusted to produce films containing the same amount of A. The extent of fibrinogen fouling on these now comparable films was measured with surface plasmon resonance imaging to evaluate if the actual fabrication method affects a film's fouling resistance. Furthermore, electrochemically induced reductive desorption was used to indicate any presence of islanding and to evaluate film stability.

**BI-TuP10 Specific versus Non-Specific Protein Adsorption: Effects of Chain Length and Tailgroup in Functionalized Poly(ethylene glycol)-Terminated Self-Assembled Monolayers**, *P. Buecker, M. Himmelhaus,* ***R. Dahint***, University of Heidelberg, Germany

In recent years, substantial efforts have been made to develop surface coatings which limit or even suppress non-specific adsorption of proteins. While for many technological applications the surfaces are designed to exclusively repel proteins, an even more complex situation exists in biomedical analysis, where immobilized probe molecules, e.g. antibodies, are used to specifically bind target proteins from solution. Here, the coating must fulfil a two-fold function: (i) effectively suppress non-specific adsorption processes, which may both result in false interpretation of binding events and significantly lower the detection limit of the analytical techniques, and (ii) additionally facilitate the integration of the probe molecules without loss of non-specific protein repulsion. Hereby the question arises, to what extent efficient immobilization of probe molecules and suppression of non-specific interactions can actually be achieved with a single material as the repulsion mechanism inhibiting non-specific adsorption of biomolecules might also compromise the integration of the probes. In a recent study we have shown that COOH-functionalized poly(ethylene glycol) (PEG) alkanethiolate self-assembled monolayers (SAMs) [$HS-(CH_2)_{11}-(OCH_2CH_2)_n-COOH$] with a mean number, n, of 33 EG units suppress non-specific protein adsorption while facilitating covalent coupling of antibodies via the terminal COOH-group.[1] We now synthesized the same type of molecule with various EG chain lengths (n = 13-40) and different tailgroups (-OH, $-NH_2$, and -COOH), and compared both the antigen binding capacity and the protein resistance of the corresponding SAMs by ellipsometry, X-ray photoelectron spectroscopy (XPS), infrared spectroscopy (FT-IRRAS), and enzyme-linked immunosorbent assays (ELISA). It is observed that protein repulsion depends on the tailgroup selected and increases with increasing EG chain length. In parallel, however, the maximum amount of probe molecules that can be coupled to the films significantly decreases with enhanced EG content, thus, reducing the antigen binding capacity of the films. The results, therefore, show that for bioanalytical applications the number of EG units has to be properly adjusted in order to obtain an optimum signal-to-noise-ratio. The best performance has been observed for a chain length of about 30 EG moieties.

[1] S. Herrwerth et al., Langmuir 2003, 19, 1880.

**BI-TuP11 Quantitative Analysis of Angular Resolved XPS-Measurements: Influence of Inaccuracies in Take-Off and Acceptance Angle**, ***C. Van der Marel***, *J.H.M. Snijders, D.D.C.A. Van Oers, E.P. Naburgh*, Philips Research - MiPlaza, The Netherlands

For quantitative analysis of Angular Resolved XPS-measurements the take-off angle of the detected electrons is an important input parameter. A device has been developed that allows measurement of the real take-off angle as a function of the tilt angle of the sample. The acceptance angle of the detector is also determined using this device. The device has been tested extensively in two commercial XPS-instruments (Quantera from ULVAC – PHI); small but significant deviations have been found between real and set values of the take-off angles. To investigate the influence of the acceptance angle and of inaccuracies in the take-off angles several series of samples were

analyzed. The samples consisted of a homogeneous substrate coated with a thin organic layer (e.g. phosphonic SAM-layers on Al2O3, alkyl-thiols on copper). Quantitative analysis of the XPS-results provided values for the layer thickness of the organic layer, for the coverage and for the "real" atomic concentrations in substrate and in the organic layer. In the lecture it will be shown that the use of accurate values of the acceptance and the take-off angles is a prerequisite to obtain consistent results.

**BI-TuP12 Improved Osseointegration of a Novel, Hydrophilic Ti Surface: Early Events**, *A. Molenberg, **S. Berner**,* Institut Straumann AG, Switzerland, *F. Schwarz, M. Herten,* Heinrich Heine University, Germany, *M. de Wild, M. Wieland*, Institut Straumann AG, Switzerland

The performance of bone-related implants is strongly connected with the properties of the implant surface. Particularly surface topography and chemistry both influence initial wettability and peri-implant bone apposition of implants. Sandblasted and acid etched (SLA) titanium surfaces are initially hydrophobic due to microstructuring and adsorption of hydrocarbons and carbonates. The purpose of this study was to investigate initial hydrophilicity and to examine tissue reactions to a newly developed chemically modified implant surface called SLActive. Implantation of SLActive and SLA implants was performed bilaterally in dogs. The animals were sacrificed after a healing period of 1, 4, 7 and 14 days, respectively. Peri-implant tissue reactions were assessed in nondecalcified tissue sections using conventional histology (Toluidine blue-TB and Masson Goldner Trichrome stain-MG) and immuno¬histochemistry using monoclonal antibodies to transglutaminase II (TG) (angiogenesis) and osteocalcin (OC) (mineralization). Bone density (BD) and bone to implant contact (BIC) were assessed histomorphometrically. Immunohistochemical staining for TG revealed a direct correlation between angiogenesis and new bone formation, which was clearly identifiable after 7 days by means of increasing BD, BIC and OC values. After 14 days, SLActive surfaces seemed to be surrounded by a firmly attached mature, parallel-fibered woven bone. The study clearly reveals an enhanced bone formation for the hydrophilic SLActive surface and thus a reduced healing time.

**BI-TuP13 Solution Deposited poly(N-isopropyl acrylamide) Films Optimized for Mammalian Cell Release**, ***J.A. Reed**, M. Bore, L.K. Ista, G.P. Lopez, H.E. Canavan*, University of New Mexico

Poly(N-isopropyl acrylamide), or pNIPAM, is a smart polymer that is of great interest in the bioengineering community. Above its lower critical solution temperature (LCST), the polymer is relatively hydrophobic, and mammalian cells grown on pNIPAM-grafted surfaces act in a similar fashion as those grown on typical tissue culture polystyrene (TCPS): they proliferate into a confluent sheet. Below the LCST (i.e., room temperature) the polymer physically changes, becoming hydrophilic and swelling. Furthermore, when the temperature of the cell culture is dropped below the LCST of the polymer, the cells will detach and can be harvested for tissue engineering applications. This is in contrast to cells grown on TCPS alone, which will not detach using a temperature drop, instead requiring enzymatic digestion (via trypsin) or mechanical scraping to remove them. In this work, we present a novel, solution-based technique to generate pNIPAM surfaces from pNIPAM and tetraethyl orthosilicate (spNIPAM). The composition of the films (i.e., weight % of pNIPAM) was optimized for reversible cell adhesion by observing the thermoresponse, surface chemistry, and cell release characteristics. Characterization of the surface chemistry of the spNIPAM substrates was done using X-ray photoelectron spectroscopy (XPS), interferometry, and contact angle measurements). Characterization of the cell-releasing behavior of the spNIPAM substrates was determined by observing the ability of surfaces to release confluent bovine aortic endothelial cells (BAECs). From these results, we conclude that the fastest, most reliable release of BAECs occurred on surfaces spin coated with a solution of .35wt% pNIPAM/TEOS.

**BI-TuP14 Nanometer-scale Cell Adhesive Patches for the Control of Cellular Functions**, ***U.A. Martinez**, T.C. Gamble, G.P. Lopez, E.L. Dirk*, The University of New Mexico

The control of cellular functions will find many applications in the development of new materials for biomedical applications such as tissue engineering. However, for this to be possible, understanding the cell-material interactions is essential. In this work, interferometric lithography was used to create nanometer-scale patterned substrates. Interferometric lithography is a method for fabricating spatially consistent periodic patterns with the use of the interference of two coherent optical beams. The interfering beams produce a standing wave that can be recorded in a photoresist making equally spaced channels. The substrate is then rotated 90° and exposed again creating photoresist pillars. After exposure, the substrates are coated with a 30 nm layer of gold, using a 3 nm layer of chromium to enhance adhesion. An acetone lift-off procedure removes the photoresist pillars, leaving uncoated patches throughout the gold-coated surface. Self-assembled monolayers are formed on the gold-coated areas using thiols of poly(ethylene glycol) [PEG], a biologically inactive molecule that inhibits protein adsorption. Fibronectin, a protein that enhances cellular adhesion, is adsorbed onto the uncoated regions rendering cell adhesive patches. A double exposure technique has also been developed to obtain a two-fold increase in the pitch while holding the same patch size. For example, a pitch of 300 nm with a 100 nm patch can be increased to a 600 nm pitch while maintaining the same size of the patch. These nanopatterned cell adhesive substrates offer a method to systematically manipulate cellular attachment to further control cellular functions such as spreading, viability and differentiation.

**BI-TuP15 Competitive Protein Adsorption to Biomaterials and the Dependence on Protein Concentration**, ***M. Holmberg**, X. Hou*, Technical University of Denmark

Quantitative analysis of protein adsorption to artificial materials is of importance in evaluating the potential of biomaterials. Proteins have a central role in all living organisms and are present in basically all parts of the human body and the first thing to happen when an artificial material is introduced into a human body, or another biological system, is unspecific adsorption of proteins onto the surface of the material. Most studies on blood protein adsorption to polymer surfaces reported in the literature are performed from low concentration (≤1 mg/ml) single protein solutions. However, it is not evident that those results also are valid for protein adsorption from protein mixtures with high protein concentration (≥10 mg/ml). When biomaterials are introduced into a human body, thousands of different proteins, sometimes present in quite high concentrations, will come in contact with the material and there will be competitive protein adsorption on the surface of the material. By labelling proteins with different radioisotopes one can monitor the adsorption of several proteins onto surfaces simultaneously, and with this multi-labelling technique one can study competitive adsorption and use complex solutions such as human serum during adsorption experiments. Albumin and IgG (Immunoglobulin G) adsorption to both unmodified and modified PET (polyethylene terephthalate) surfaces from single protein solutions and human serum solutions has been monitored using radioactive labelling. The PET surfaces are modified through plasma polymerisation using the monomer DEGVE (diethylene glycol vinyl ether), which results in a more hydrophilic and anti-fouling surface than the PET surfaces. Results show that the detected anti-fouling quality of DEGVE surfaces is influenced by the concentration of the protein solution used. Albumin and IgG adsorption from human serum dilutions corresponding to 0.1% human serum shows that the albumin and IgG adsorption is significant lower on the DEGVE surface than on the PET surface, and that this antifouling characteristics of DEGVE surfaces can be stable for at least 24 hours. However, performing the same experiment, including the same rinsing procedure, with ≥10% human serum solutions, the detected albumin and IgG adsorption to DEGVE approaches the adsorption detected on unmodified PET. Thus, evaluation of biomaterials should be performed in an environment similar to the one the material is intended to operate in.

**BI-TuP16 Scanning Tunneling Microscopy Studies of Photoactive Yellow Protein Anchored to the Thiol Modified Au(111) Surface**, ***I.I. Rzeznicka**,* RIKEN, Japan, *G.W.H. Wurpel,* Utrecht University, The Netherlands, *M. Bonn,* FOM Institute for Atomic and Molecular Physics (AMOLF), The Netherlands, *M.A. van der Horst, K. Hellingwerf,* University of Amsterdam, The Netherlands, *S. Matsunaga,* The University of Tokyo, Japan, *T. Yamada,* RIKEN, Japan, *M. Kawai*, The University of Tokyo, Japan

Adsorption of proteins on metal surfaces and their interactions present an important technological challenge in the field of biotechnology and construction of biomolecules-based functional devices. The process of protein adsorption is very complex and depends on surface properties, protein structure and its chemical composition. On Au surfaces, covalent bonding to the surface can in principle be easily achieved by utilizing unbound sulfur-containing amino acids resulting in the formation of strong Au-S bond, in analogy to the well known thiol chemistry. We studied adsorption of photoactive yellow protein (PYP) protein on Au(111) surface by scanning tunneling microscopy (STM) in air and in the electrochemical environment. The results show that cysteine-containing mutant of PYP does not adsorb on the bare Au(111) surface. Stable, densely packed protein layers were observed after protein immobilization via amide bond formation onto self-assembled monolayers (SAMs), consisting of 3-mercaptopropanoic acid (3-MPA) and 11-mercaptoudecanoic acid (11-MUA) with the use of carbodiimide and N-hydroxysuccinimide. Layers of PYP prepared in this way show fluorescence activity visualized by fluorescence microscopy, indicating that the protein retains its photoactive function upon adsorption. We will also present our attempts to detect a photocurrent generated by laser illumination of PYP layers.

**BI-TuP17 Fabrication of Micro-Templates for the Control of Bacterial Immobilization**, ***Y. Miyahara***, *N. Saito, O. Takai*, Nagoya University, Japan

Well-defined micro-patterns of bacteria are significant as a fundamental technique for biosensor arrays utilizing rapid detection of infectious diseases and toxic compounds. Many researchers have studied control methods to fabricate the micro-patterns. However, in these researches, many processes are required for the immobilization and the regioselectivity is not so good. In this study, we aim to fabricate well-defined micro-patterns of bacteria using two types of micro-patterned templates and to elucidate the adhesion behaviors of several bacteria onto the templates from viewpoint of surface topology and chemical properties. Super-hydrophobic/super-hydrophilic and super-hydrophobic/polyethylene glycol (PEG) micro-patterns were fabricated as the templates. In the case of super-hydrophobic/super-hydrophilic patterns, the difference of surface energy in the respective regions differs greatly. The difference could have a great effect on the adhesion of bacterial. In the case of super-hydrophobic/PEG micro-patterns, PEG surface generally avoid the adhesion of bacteria due to volume exclusion effect of PEG based on the structural fluctuation. Super-hydrophobic surface was prepared by microwave plasma enhanced chemical vapor deposition (MPECVD) from trimethylmethoxysilane (TMMOS). Super-hydrophobic/super-hydrophilic micro-patterns were fabricated by irradiating the super-hydrophobic surface with vacuum ultra violet (VUV) light through a stencil mask. In the case of Super-Hydrophobic/PEG micro-patterned surfaces, PEG surfaces were fabricated by reacting COOH groups of methoxypolyethylene glycol propionic acid with $NH_2$ groups of $NH_2$-terminated self assembled monolayer in ion-exchanged water. The super-hydrophobic regions were fabricated by MPECVD thorough a stencil mask. Bacteria were cultured on the respective templates in the incubator controlled under the $CO_2$ concentaration of 5 % at 37 $^{o}$C. After culture, bacteria were observed by phase-contrast microscope. The surfaces were characterized by XPS and FT-IR. As a result, in the case of super-hydrophobic/super-hydrophilic micro-patterns, some bacteria (Escherichia coli, Bacillus subtilis) were adhered to only super-hydrophobic regions. But others bacteria (Pseudomonas stutzeri, Pseudomonas aeruginosa) were not separated well. Their results attribute bacterial charge, cell division rate and adhesion time lag between super-hydrophobic regions and super-hydrophilic regions.

**BI-TuP18 Kinetic Study on Protein Adsorption on Polyelectrolyte Brush Surface**, ***H. Tatematsu***, Nagoya University, Japan, *T. Fujima*, Musashi Institute of Technology, Japan, *N. Saito, O. Takai*, Nagoya University, Japan

Polymer chains grafted in the high density stretch perpendicularly by repulsive interaction. This is a so-called brush. The various functional groups can be also introduced into polymer brush as lateral chains. The 3-dimensional structure was varied by the type of lateral chains. On spherical polyelectrolyte brush (PEB), strong adsorption of protein takes place at low ionic strength whereas less protein is adsorbed at the high ionic strength.[1] In addition, protein structure and character remain via adsorption.[2] These properties can be applied to improvement of biocompatibility and Drug Delivery System (DDS). However, the kinetics of adsorption on normal PEB surface has not been understood well. Thus, in this study, we aimed to investigate the kinetics of protein adsorption on PEB. Polystyrene brush (PSB) surface was fabricated by reacting polystyrene terminated by a trichlorosilane (PS-$Cl_3$) group with silanol (Si-OH) groups on the silicon wafer. PSB brush was converted to polystyrene sulfonate sodium salt brush (PSSB) by sulfonation and neutralization. In protein adsorption experiment, the solution of Bovine Serum Albumine (BSA) in phosphate buffer saline (PBS) was used. NaCl aqueous solution adjusted to various concentrations was added to the stock solution in order to extract the effect of ionic strength. Protein adsorption was evaluated by Fourier transform infrared spectroscopy (FT-IR) and the evanescent adsorption spectroscopy (EOS). The proteins on the surfaces were observed by atomic force microscope (AFM). Relationship between the amount of adsorption and the ion strength on normal PSSB surface differs from that on spherical PSSB surface in previous reports. This would originate from structural configuration of PEB. Finally, we propose a kinetic model of the adsorption on PSSB based on the variation of adsorption against time and ion strength by FT-IR, EOS and AFM.

[1] A. Wittemann and M. Ballauff, Phys. Chem. Chem. Phys., 2006, 8, 5269-5275
[2] B. Haupt, Th. Neumann, A. Wittemann, and M. Ballauff, Biomacromolecules 2005, 6, 948-955

**BI-TuP19 Surface Characterization of PEG-like Film Made by Using Ethylene Glycol Precursor and Capacitively Coupled Plasma Chemical Vapor Deposition**, ***C. Choi***, *S. Lee, D. Jung,* Sungkyunkwan University, Korea, *D.W. Moon, T.G. Lee*, Korea Research Institute of Standards and Science (KRISS)

Polyethylene-glycol (PEG) is widely used for various biological applications because of its anti-fouling property for protein and cells, and non-toxicity in the human body. Since the PEG thin film should have the properties of reproducibility and adhesiveness on various substrates, in this work, plasma-polymerized ethylene glycol (PPEG) thin film was deposited on a glass surface by using the capacitively coupled plasma chemical vapor deposition (CCP-CVD) method and ethylene glycol as a precursor. The PPEG thin films were characterized by using contact angle measurement, X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) along with a principal component analysis (PCA). The PPEG surface was controlled in a reproducible manner as a function of sample bias plasma power and was correlated with the surface chemical composition. Of interest, we found that PPEG thin film surfaces deposited at low plasma power were similar in chemical composition to the PEG polymer surface. In addition, by using the fluorescence detection method, we found that the PPEG surface showed an anti-fouling property of immunoglobulin G protein, which was tagged by fluorescein isothiocyanate. Our results showed that this PEG-like PPEG surface would be useful for protein chip applications.

**BI-TuP20 Determination of Standard-State Adsorption Free Energy for Peptide-Surface Interactions by SPR Spectroscopy without Peptide-Peptide Interaction Effects**, ***R.A. Latour***, *Y. Wei*, Clemson University

As an approach to gaining fundamental insights into the thermodynamics of protein-surface interactions, we are quantitatively determining the standard state free energy of peptide-surface interactions using a host-guest peptide model in the form of TGTG-X-GTGT, where the T (threonine) and G (glycine) flanking sequences are the host residues and X represents a variable guest residue. Alkanethiol self-assembled monolayers (SAMs) with a broad range of polymer-like functionalities are being used as the adsorbent surfaces. With this experimental model, we are able to determine contributions of individual mid-chain amino acid residues on peptide adsorption behavior. The most common way of determining the standard state free energy of adsorption of a peptide to a surface is by use of the Langmuir equation fitted to an adsorption isotherm. This method, however, has the inherent problem of the influence of peptide-peptide interactions at the interface. These effects can substantially affect the shape of the isotherm, leading to errors in the calculated values of the standard state free energy of adsorption. We have developed a new approach to solve this problem and provide accurate, quantitative measurements of the standard state adsorption free energy using surface plasmon resonance (SPR) spectroscopy based on chemical potential relationships vs. the classical Langmuir equation. In addition, we have also developed a very simple, automated method of accounting for bulk shift effects, which can be problematic when doing SPR adsorption studies. These novel methods will be presented along with results for a series of peptide adsorption systems using these methods.

# Authors Index

## Bold page numbers indicate the presenter