Biomaterial Interfaces
Room 311 - Session BI+SS-FrM

Biomaterials Surface Characterization
Moderator: S.L. McArthur, University of Sheffield, UK

8:20am **BI+SS-FrM1 Charge Injection Barriers and Gap States at the L-Cysteine/Au Interface**, ***M.M. Beerbom***, *R. Gargagliano, R. Schlaf,* University of South Florida

Protein/inorganic materials interfaces are interesting for many bio-engineering applications such as bio-sensors or molecular electronic devices. L-cysteine is particularly interesting since it can form self-assembled monolayers on gold due to its thiol-group. In our experiments we investigated the L-cysteine/Au interface using x-ray and ultraviolet photoelectron spectroscopy (XPS, UPS) in combination with a multi-step deposition procedure. PE-spectra obtained after each deposition step yielded a data set allowing the determination of charge injection barriers at the interface, as well as the characterization of the chemical interaction. The L-cysteine deposition was carried out in a glove box directly attached to the PES system enabling contamination-free measurements. Our results indicate the formation of an interface state within the HOMO-LUMO gap due to the thiol-Au interaction.@footnote 1@ This is supported by control experiments on L-cysteine/graphite interfaces, which did not show the formation of this gap state. @FootnoteText@ @footnote 1@M.M. Beerbom, R. Gargagliano and R. Schlaf: "Determination of the Electronic Structure of Self-Assembled L-cysteine/Au Interfaces Using Photoemission Spectroscopy", Langmuir Articles ASAP), (2005).

8:40am **BI+SS-FrM2 Injection into Vacuum and Alignment of Biological Molecules for Electron Diffraction**, ***D. Starodub***, *R.B. Doak, J.C.H. Spence, U. Weierstall, K. Schmidt, G. Hembree,* Arizona State University

Resolving the protein secondary structure (folding),critical for its functionality, is a demanding task, especially for the proteins, which cannot be easily crystallized. Recently it was proposed to collect diffraction patterns dynamically from an array of biological molecules embedded in submicron water droplets, consecutively traversing the intersection of a focused 50 keV electron beam and a polarized 100 W laser beam.@footnote 1@ The latter aligns the molecules due to field interaction with a dipole moment induced in the molecule with anisotropic polarizability tensor.@footnote2@ We show experimental results on generation of monodispersed microdroplets via growth of Rayleigh instability, their injection into high vacuum, evaporation and cooling. The limitations on the droplet size and temperature for a given jet source configuration are obtained. Rotational relaxation of the spherical (small protein) and rodlike (tobacco mosaic virus) biomolecules to thermal fluctuations about the equilibrium orientation is considered in viscous and free molecular flow regimes, and optimal conditions for alignment, sufficient to obtain sub-nanometer resolution in diffraction, are derived. We also consider adiabatic effects of different spatial profiles of laser beam intensity and droplet velocity on final oscillation states of a biomolecule. Supported by NSF funding SGER DBI-0429814. @FootnoteText@ @footnote1@J.C.H. Spence and R.B. Doak, Phys. Rev. Lett. 92, 198102(2004). @footnote2@J.C.H. Spence, K. Schmidt, J. Wu, G. Hembree, U. Weierstall, R.B. Doak, P. Fromme. Acta Cryst. A61, 237(2005).

9:00am **BI+SS-FrM3 Chemical Interaction Analysis of Adhesive Biomaterial-Hard Tissue Interfaces**, ***Y. Nakayama***, Toray Research Center, Inc., Japan*; Y. Yoshida, K. Suzuki,* Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan*; B. Van Meerbeek,* Catholic University of Leuven, Belgium

Adhesion to human hard tissue plays an important role in both dental and orthopedic therapies. Synthesized adhesive biomaterials made of polymer, as well as of functional monomer, have been developed. Among such adhesive biomaterials, those capable of forming chemical bond with hard tissue such as dentin, enamel or hydroxyapatite (HAp) are more appreciated for achieving more durable adhesion. Evidence of chemical bonding at biomaterial-hard tissue interfaces was recently reported for interfaces between polyalkenoic acid and enamel or HAp with the use of high resolution X-ray Photoelectron Spectroscopy (XPS).@footnote 1@ Preparation of ultrathin biomaterial molecular layer on enamel or HAp substrate enabled high resolution XPS spectrum to distinguish carboxylic carbons with chemical bond to HAp from those without chemical bond at the interface. Chemical interaction of carboxylic acids with HAp was analyzed and adhesion-decalcification concept (AD concept) was proposed.@footnote 2@ According to the AD concept, the less soluble the calcium salt of the acidic molecule, the more intense and stable the molecular adhesion to a hydroxyapatite-based substrate. Subsequently, adhesive performance of functional monomers, such as those containing dihydrogen phosphate or carboxylic acid, with HAp and dentin was studied.@footnote 3@ In this presentation, we will report chemical interaction analysis of functional monomer-hard tissue interfaces by utilizing high resolution XPS and relating analytical data. @FootnoteText@ REFERENCES:@footnote 1@ Y.Yoshida, B.Van Meerbeek, Y.Nakayama, J.Snauwaert, L.Hellemans, P.Lambrechts, G.Vanherle, K.Wakasa: J.Dent.Res., 79, 709 (2000).@footnote 2@ Y.Yoshida, B.Van Meerbeek, Y.Nakayama, M.Yoshioka, J.Snauwaert, Y.Abe, P.Lambrechts, G.Vanherle, M.Okazaki: J.Dent.Res., 80, 1565(2001) .@footnote 3@ Y.Yoshida, K.Nagakane, R.Fukuda, Y.Nakayama, M.Okazaki, H.Shintani, S.Inoue, Y.Tagawa, K.Suzuki, J.De Munck, B.Van Meerbeek: J.Dent.Res., 83, 454(2004) .

9:20am **BI+SS-FrM4 Evaluation of Residues on Implant Surfaces by X-ray Photoelectron Spectroscopy**, ***V. Frauchiger***, *R. Luginbuehl,* Dr. H.C. Robert Mathys Foundation

Success of modern implants does not depend on the proper material choice only, but also on the surface chemistry and the proper removal of the ubiquitous present surface contaminations. There are many contemporary analytical methods that permit the qualitative and quantitative analysis of picogram amounts, but only a few methods are suitable for a direct assessment without a special preparation of the implants or the chemical extraction of residues. We applied X-ray photoelectron spectroscopy to assess the cleanliness of osteosynthesis plates and hip endoprosthesis implants. In addition, specially designed test coupon surfaces were used to simulate different surface textures. The samples were soiled with exact amounts of two model process adjuvants (MPA). The first one was based on a mixture of organic compounds used in lubricants and cutting fluids while the second one contained inorganic particles used in blasting processes. The cleaning efficacy of an industrial cleaning process in aqueous media and with sonication was tested. Imaging XPS was applied to localize critical contamination on the surfaces and small area analysis was used to identify the organic residues. In addition to XPS, GC-MS and gravimetric analysis were carried out as complementary techniques. Principal component analysis was used to establish a sensitivity at the ng/cm@super 2@ level by calculating the ratio between the bulk implant material and the carbon or other MPA specific elements. Cleaning tests revealed that the organic residues are completely removed upon proper choice of cleaning conditions and detergents. Residual inorganic particles were found on many samples with a blasted surface texture. The particles were removed only by special treatment of the samples.

9:40am **BI+SS-FrM5 Characterisation of Analyte / Matrix Interaction for MALDI / TOF Targets Using Spatially Resolved X-ray Photoelectron Spectroscopy**, ***A.J. Roberts***, *D.J. Surman, S.J. Hutton,* Kratos Analytical Ltd, UK*; M. Resch,* SDG, Germany*; E. Raptakis, O. Belgacem,* Kratos Analytical Ltd, UK

Matrix-assisted laser desorption/ionisation (MALDI) is now an established technique for mass spectrometry of proteins and peptides. Different matrix-analyte preparation protocols have been shown to influence the desorption or ablation process resulting in either high or low metastable fragmentation. It has been speculated that following laser ablation the velocities of the analyte and matrix can be regarded as a valuable and meaningful characteristic of the MALDI process. However, the interaction and distribution of the analyte with respect to the matrix is poorly understood. Here we present a study of the distribution of a fluorinated peptide as a function of matrix material using imaging x-ray photoelectron spectroscopy (XPS). Both the lateral and depth distribution is investigated to draw conclusions on the incorporation of the analyte in the matrix.

10:00am **BI+SS-FrM6 In Situ Sum Frequency Generation Characterization of Adsorbed Alpha-helical Peptides**, ***N.T. Samuel***, University of Washington*; K. McCrea,* Polymer Technology Group*; L.J. Gamble,* University of Washington*; R.S. Ward,* Polymer Technology Group*; D.G. Castner,* University of Washington

Controlling and characterizing the structure of adsorbed biomolecules is important for applications in diagnostics, tissue engineering and nanobiotechnology. Our previous studies showed that peptides with well-defined sequences of lysine (K) and leucine (L) amino acids spontaneously adsorb onto hydrophobic substrates with an alpha-helix secondary structure. The present study characterizes the adsorption of the LK peptides onto the surface through two approaches - immersing the

hydrophobic substrate through the air-water interface (AWI) and avoiding the AWI. When the LK peptide is adsorbed avoiding the AWI a time-dependent change in the amide I intensity is observed. However, X-ray Photoelectron Spectroscopy (XPS) analysis showed no significant time dependence of the nitrogen surface composition. Similarly, the CH region of the SFG spectrum shows no time-dependence. These results indicate that the amide I SFG spectrum follows the time-dependent ordering of the peptide molecules on the hydrophobic surface. Results from site-specific labeling of the alpha-helix LK peptide molecules with deuterated leucine residues showed it was possible to follow interactions of one amino acid residue of the peptides with the surface.

10:20am **BI+SS-FrM7 Molecular Level Studies on Interfacial Protein and Peptide Structures Using SFG**, ***Z. Chen***, University of Michigan

Molecular structures of interfacial proteins and peptides play important roles in biocompatibility, anti-biofouling control, membrane protein function, and anti-microbial peptide behavior. Sum frequency generation (SFG) vibrational spectroscopy has been applied to investigate molecular structures of proteins and peptides at the solid/liquid interface in situ. A thin film model was adopted to interpret SFG spectra. Isotope labeled method was introduced in the SFG studies. SFG results demonstrate that C-H stretching and amide signals can elucidate structures of hydrophobic side chains and secondary structures of interfacial peptides and proteins respectively. Detailed correlations between SFG amide I signals and secondary structures of interfacial proteins and proteins have been deduced. Various peptides and proteins including several anti-microbial peptides, albumin, lysozyme, fibrinogen, and factor XII (FXII) have been studied. Time-dependent structural changes of adsorbed proteins at the solid/liquid interface have been monitored. In addition, SFG chiral spectra were collected from interfacial peptides and proteins, which provide additional measurements to determine structures of these proteins and peptides. We also showed that combinations of linear vibrational spectroscopy such as attenuated total reflection Fourier transform infrared spectroscopy with nonlinear vibrational spectroscopic techniques such as SFG and four-wave mixing provide more detailed structure of surfaces/interfaces than that from a single technique.

10:40am **BI+SS-FrM8 Using Ellipsometry and Textured Surfaces to Enhance Infrared Response of Adsorbed Biomaterials**, ***D.W. Thompson***, *J.A. Woollam,* University of Nebraska-Lincoln

Infrared spectra are of interest for numerous applications because of the chemical bond information present in the absorption characteristics, but obtaining meaningful infrared spectra from monolayers adsorbed to surfaces can be difficult because of the small amount of material being probed. For instance, it is often of interest to probe adsorbates on a surface after exposure to a protein solution. Use of textured (patterned) surfaces to increase the mass of material sensed is expected to enhance these spectra. Here the infrared ellipsometric enhancement is calculated for a layer of adsorbate on a number of proposed nanostructured surfaces to predict which is most advantageous for obtaining infrared spectra. The approach used here could also be applied to other adsorbates by optimizing the pattern dimensions for different sizes. It also works for visible spectroscopy as long as pattern dimensions are significantly smaller than the wavelength. The effect of using these structures (rods, wells, and trenches) is compared to the response of flat metal or dielectric surfaces over a range of incidence angles of the infrared beam. Predicted sensitivities are based on the calculated effect of adsorbate on intensities in an ellipsometric measurement. Trench structures appear to have significant advantages both in sensitivity and ability to distinguish adsorbed species orientation.

11:00am **BI+SS-FrM9 Understanding the Elasticity of Fibronectin Fibrils: A Single Molecule Force Spectroscopy Study**, ***N.I. Abu-Lail***, *T. Ohashi, R. Clark, H. Erickson, S. Zauscher,* Duke University

Although fibronectin (FN) is considered to be one of the most important components of the extracellular matrix, the detailed mechanism of the elasticity of FN fibrils is still unknown. To investigate the molecular origin of FN fibril elasticity we performed single molecule force spectroscopy (SMFS) measurements on a recombinant 1-8FN-III protein construct that contained two green fluorescent protein (GFP) domains spliced in-between the 3FN-III and 4FN-III domains. The relative strengths of both domains were investigated over a wide range of pulling rates (50 nm/s to 1745 nm/s). FN-III domains were distinguished from GFP domains based on their characteristic unfolding distance signature. We found that the mechanical stability of both domains was similar and that the unfolding forces of both domains were linearly related to the logarithm of the pulling rate. An extrapolation of the unfolding forces to small pulling rates showed that the force required to unfold the FN-III domains and GFP domains were undistinguishable and on the order of physiological forces (~10 pN). Our results, combined with earlier fluorescence resonance energy transfer (FRET) studies performed on the same recombinant proteins, suggests (1) that the FN-III domains are most likely bent and looped into a compact conformation in the cell culture and (2) stretching extends their conformation while the domains remain mostly folded.

11:20am **BI+SS-FrM10 Protein-Solvent Interactions in Surface-Grafted ELPs Measured by Single Molecule Force Spectroscopy**, ***A. Valiaev***, *D.W. Lim, S. Schmidler, A. Chilkoti, S. Zauscher,* Duke University

Stimulus-responsive biomolecules attract significant research interest due to their potential applications in areas such as drug delivery, molecular motors and nanoscale sensors. Here we present our results of the conformational and hydration behavior of surface grafted elastin-like polypeptides (ELPs), measured by single molecule force spectroscopy. ELPs are stimulus-responsive polypeptides that contain repeats of the five amino acids Val-Pro-Gly-Xaa-Gly (VPGXG), where Xaa is a guest residue, and undergo an inverse phase transition in response to an environmental stimulus, such as a change in temperature. Our results suggest that single-molecule force spectroscopy can be used to quantify the effect of the type of guest residue, pH or ionic strength on molecular conformation and elasticity. By fitting ELP force-extension data to a freely jointed chain model, using our newly developed data analysis approach, we showed that we can resolve differences in Kuhn segment lengths as small as 0.03 nm; i.e., differences that are about an order of magnitude smaller than those previously reported. The observed force-extension behavior at intermediate and large extensions supports a phenomenological model that describes ELPs as kinetically mobile and disordered macromolecules. Importantly we find that molecular elasticity upon extension arises both from a deformation of the polypeptide backbone and from hydrophobic polymer-solvent interactions. Our observations here agree with recent MD simulations which suggest that hydrophobic hydration of side-chains plays an important role for elasticity and provides the molecular basis for the inverse temperature transition behavior.

11:40am **BI+SS-FrM11 Developments of Flexible Tethers to Measure Antibody-Antigen Interactions using AFM**, ***Z. Suo***, *F. TerÃ¡n Arce, R. Avci, E. Smith, K. Thiltges, B. Spangler,* Montana State University

Functionalization of an AFM tip surface with covalently bound flexible tether molecules is of special interest because such a flexibility is necessary to measure, in a controllable fashion, the receptor-ligand binding events in the physiological environment of the biomolecules. However, the interpretation of the experimental data is often obscured by and confused with the nonspecific binding events between the substrate surface and the so functionalized AFM tip. Effective methods must be developed to eliminate and/or to identify these nonspecific binding events. To achieve these objectives we employed low densities of varying-length poly(ethylene glycol) (PEG) units grafted onto gold-coated AFM tip surfaces. These tethers were covalently linked to the antibodies of interest, in this case anti-cytochrome c. It was necessary to pacify the uncovered portions of the AFM tip in order to block the nonspecific tip-surface interactions. This was achieved by using the flowers-in-the-meadow concept: by mixing a self-assembled monolayers of small molecular size hydroxyl-terminated PEG unit (meadow) with the specifically terminated larger molecular size PEG unit (flower) as described above. AFM force-extension measurements using such a tip conducted on mica substrate covered with cytochrome c resulted in force and length distributions which are consistent with the tether lengths employed. The pH value and the ionic strength of the buffer have considerable influence on the binding events between the AFM tip and the surface covered with cytochrome c. We will present results covering these topics as well as the role of the coupling chemistry between the end terminals of a PEG molecule and the anti-cytochrome c and the AFM tip on the efficiency of antibody-antigen recognition events.

Author Index

Bold page numbers indicate presenter