Biomaterial Interfaces
Room: Taos A - Session BI2+AS-TuA

Combining Techniques for Biointerface Characterization
Moderator: L.J. Gamble, University of Washington

4:00pm **BI2+AS-TuA7 Spatial and Depth Characterisation of Immobilised Biomolecules on Surfaces**, ***G. Mishra***, *A.J. Roberts,* Kratos Analytical Ltd., UK*, D.J. Surman,* Kratos Analytical Ltd.*, S.L. McArthur*, Swinburne University of Technology, Australia

4:20pm **BI2+AS-TuA8 Measuring the Orientation of Electrostatically Immobilized Proteins by Time-of-Flight Secondary Ion Mass Spectrometry and Sum Frequency Generation: From a Model Protein G B1 System to Cytochrome**, ***J.E. Baio***, *T.M. Weidner, L. Baugh, P.S. Stayton, L.J. Gamble, D.G. Castner*, University of Washington

The ability to orient proteins on surfaces to control exposure of their biologically active sites will benefit a wide range of applications including protein microarrays and biomaterials that present ligands to bind cell receptors. As methods to orient proteins are developed, techniques are required to provide an accurate picture of their orientation. Since no single technique provides a high-resolution image of surface-bound proteins, combinations of surface analytical techniques are required. In this study, we have developed a model system based on the electrostatic immobilization of a small rigid protein (Protein G B1 domain, 6kDa) to further develop the capabilities of time-of-flight secondary ion mass spectrometry (ToF-SIMS) and sum frequency generation (SFG) spectroscopy as tools to probe the orientation of surface immobilized proteins. A Protein G mutant (D4) exhibiting net positive and negative charges at either end (for pH 6-8) was produced by neutralizing four negatively charged residues closest to the end of the protein (Asp to Asn or Glu to Gln mutations). These mutants were then immobilized onto NH_3^+ and COO^- terminated self assembled monolayers (SAMs) to induce opposite end-on orientations. ToF-SIMS data from the D4 variant on both NH_3^+ and COO^- SAMs showed intensity differences from secondary ions originating from asymmetric amino acids (Asn:70, 87, and 98m/z; Met:62m/z; Tyr:107 and 136m/z at the N-terminus. Leu:86m/z at the C-terminus). For a more quantitative examination of orientation, we developed a ratio comparing the sum of the intensities of ions stemming from residues at either end of the protein. The 50% increase in this ratio, observed between the NH_3^+ and COO^- SAMs, indicated opposite orientations of the D4 variant on the two different surfaces. In addition, SFG spectral peaks characteristic of ordered α-helix ($1645cm^{-1}$) and β-sheet (1624 and $1675cm^{-1}$) elements were observed, with a phase that indicated a predominantly upright orientation for the α-helix, consistent with an end-on protein orientation. We then moved from this model system and extended this analysis to examine the change in orientation of horse heart Cytochrome c on both NH_3^+ and COO^- SAMs. The positively charged region at one end of Cytochrome c binds to the COO^- substrate while the NH_3^+ surface elicits the opposite binding orientation. Again, within the SFG spectra, ordering of the protein α-helices were confirmed by the feature at $1645cm^{-1}$ and the change in orientation, induced by the two different substrates, is confirmed by intensity differences within ToF-SIMS spectra between ions stemming from asymmetric amino acids (Glu:84 and 102m/z; Asp:72 and 88m/z).

4:40pm **BI2+AS-TuA9 NanoBio Imaging for Cardiovascular Researches**, ***D.W. Moon***, *T.G. Lee, J.Y. Lee, W. Jegal, S.W. Kim*, KRISS, Republic of Korea **INVITED**

NanoScience has been developed to meet the demands on atomic scale characterization and manipulation of materials and devices from semiconductor industries based on the scaling down law. KRISS has been trying to extend the application scope of nanoscience and technology from microelectronics to biomedical areas. Biochemical imaging of cells and tissues is a basic infra-technology in various bio-medical applications. Instead of conventional labeling methodology for bio-molecular imaging with fluorescent dyes, label-free biochemical imaging methodologies for single cells and tissues such as coherent anti-stokes Raman scattering (CARS), secondary ion mass spectrometry (SIMS), and surface plasmon resonance imaging ellipsometer (SPRIE) has been developed and integrated for new biomedical applications, especially for cardiovascular researches.

Preliminary results of nanobio imaging for cardiovascular researches will be reported on the following issues 1) Three-dimensional visualization of atherosclerotic tissue and prompt on-site analysis of chemical profiles by multiplex CARS with intracellular lipids at the single-cell level as well as crystallized cholesterol in necrotic cores. [(1)] 2) Histological Imaging based on SIMS analysis of myocardial infarction tissues. [(2)] 3) cell adhesion dynamics of human carotid smooth muscle cells and human umbilical endothelial cells on fibronectin thin films with SPRIE.

Finally, the present status and future challenges of nano-bio technology based on laser, mass spectrometry, and nanoprobe for biochemical imaging of single cells and tissues at KRISS will be discussed for practical applications in bio, medical, and pharmaceutical researches.

(1) "Multiplex coherent anti-Stokes Raman spectroscopy images intact atheromatous lesions and concomitantly identifies distinct chemical profiles of atherosclerotic lipids", Se-Hwa Kim, Eun-Soo Lee, JaeYong Lee, EunSeong Lee, Bok-Soo Lee, JeongEuy Park ,and DaeWon Moon, Circulation Research, in press (2010)

(2) "ToF-SIMS Analysis of Myocardial Infarcted Tissue", J.-W. Park, M.-J. Cha, H. K. Shon, S.-H. Kim, T. G. Lee, D. W. Moon, and K.-C. Hwang, Surface and Interface Analysis, in press (2010)

5:20pm **BI2+AS-TuA11 Determining Antibody Orientation using ToF-SIMS and Fluorescence Imaging of Affinity-generated Patterns**, ***M. Dubey***, Los Alamos National Laboratory, *F. Liu, H. Takahashi, D.W. Grainger,* University of Utah, *D.G. Castner*, University of Washington

This study assesses the capability of high-resolution surface analytical tools to distinguish immobilized antibody orientations on patterned surfaces designed for antibody affinity capture. High-fidelity, side-by-side co-patterning of protein A (antibody Fc domain affinity reagent) and fluorescein (antibody Fab domain hapten) was achieved photo-lithographically on commercial amine-reactive hydrogel polymer surfaces. This was verified from fluorescence imaging using fluorescently labeled protein A and intrinsic fluorescence from fluorescein. Subsequently, dye-labeled murine anti-fluorescein antibody (4-4-20), and antibody Fab and Fc fragments were immobilized from solution onto respective protein A- and fluorescein- co-patterned or control surfaces using antibody-ligand affinity interactions. Fluorescence assays support specific immobilization to fluorescein hapten- and protein A-patterned regions through antigen-antibody recognition and natural protein A-Fc domain interactions, respectively. Affinity-based antibody immobilization on the two different co-patterned surfaces generated side-by-side full antibody "heads-up" and "tails-up" oriented surface patterns. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) analysis, sensitive to chemical information from the top 2-3 nm of the surface, provided ion-specific images of these antibody patterned regions, imaging and distinguishing characteristic ions from amino acids enriched in Fab domains for antibodies oriented in "heads-up" regions, and ions from amino acids enriched in Fc domains for antibodies oriented in "tails-up" regions. Principal component analysis (PCA) improved the distinct ToF-SIMS amino acid compositional and ion-specific surface mapping sensitivity for each "heads-up" versus "tails-up" patterned region. Characteristic Fab and Fc fragment immobilized patterns served as controls. This provides first demonstration of pattern-specific, antibody orientation-dependent surface maps based on antibody domain- and structure- specific compositional differences by ToF-SIMS analysis. Since antibody immobilization and orientation are critical to many technologies, orientation characterization using ToF-SIMS could be very useful and convenient for immobilization quality control and understanding methods for improving the performance of antibody-based surface capture assays.

5:40pm **BI2+AS-TuA12 Molecular Depth Profiling and 3D Imaging of Biological Samples by ToF-SIMS: From Model Amino Acid Films to Real Biological Cells**, ***J.P. Brison***, *N. Wehbe,* University of Namur, Belgium*, D.G. Castner,* University of Washington*, L. Houssiau*, University of Namur, Belgium

Time-of-Flight Secondary Ion Mass Spectrometry is now routinely used to obtain molecular information about the surface of complex biological samples and biomaterials. The recent advent of cluster ion beams such as Bi_3^+ and C_{60}^+ provides enhanced sensitivity for high mass fragments, which in turn allows 2D imaging of specific biomolecules with improved sensitivity and sub-micrometer lateral resolution. Molecular depth profiling of biological samples with low chemical damage and high depth resolution (*i.e.*, < 10 nm) has also been shown to be possible by etching with cluster projectiles such as C_{60} and with low energy cesium ions. Since ToF-SIMS also allows the detection of all elements without the need of specific markers, the technique shows great potential for molecular 3D imaging of single cells and could become an inevitable complementary tool to MALDI and fluorescence microscopy for biomedical research in the near future.

However, only few examples of full 3D images of biological cells have been reported in the literature at this time. This limitation probably comes from the facts that preparing the cells for chemical analysis under UHV

environment is tedious, and that our understanding of energetic primary ions/biological matter interactions is limited. Fundamental studies of these aspects are indeed difficult during 3D imaging because the cells are complex, heterogeneous, non-flat samples with relatively unknown molecular composition.

In this work, fundamental biological matter/projectile interactions were studied by depth profiling model amino acid films under different ToF-SIMS conditions. The influence of the experimental parameters on the quality of the depth profiles was investigated by measuring the sputter rates, the depth resolutions and the intensities of the molecular ion signals with respect to the chemistry of the target (*e.g.*, arginine vs phenylalanine), the nature of the primary ion species (*e.g.*, C_{60}^+ vs Cs^+) and the bombardment conditions (*e.g.*, fluence and energy). Then the complexity of the model samples was increased by mixing several amino acids and by creating multilayer films. This approach was another step toward the analysis of real biological samples. Again, ToF-SIMS fundamentals were investigated by measuring, *e.g.*, the interface widths between the different organic layers and the signal decay due to the increasing etching fluence. Finally, optimal 3D images of single HeLa cells were acquired and were discussed based on the data obtained for the model amino acid films. The effect of the sample preparation on the quality of the images was also investigated.

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