Tuesday Afternoon, November 11, 2014

Biomaterial Interfaces
Room: 317 - Session BI+AS-TuA

Characterization of Biointerfaces
Moderator: Joe Baio, Oregon State University

2:20pm **BI+AS-TuA1 Comparative Study of the Bonding and X-ray Induced Reactions of Thiolated and Unthiolated DNA Adsorbed on Gold**, ***Richard Rosenberg***, Argonne National Laboratory, *J.M. Symonds*, Georgia Institute of Technology, *K. Vijayalakshmi*, Argonne National Laboratory, *D. Mishra*, Weizmann Institute of Science, Israel, *T.M. Orlando*, Georgia Institute of Technology, *R. Naaman*, Weizmann Institute of Science, Israel

High energy ionizing irradiation produces large amounts of low energy (<20 eV) secondary electrons (SEs). These electrons are produced via a cascade process following the ionization of a core (deeply bound) electron. Due to their low energy there is a high probability for the SEs to become trapped in antibonding orbitals, via resonant scattering, forming a temporary negative ion (TNI) resonance. If the lifetime of the TNI state is long enough, then bond rupture can occur by by a process known as dissociative electron attachment (DEA). There is vast literature on the role of TNI states and DEA in DNA related radiation chemistry.[1,2] Due to its high flux density, synchrotron radiation (SR) has often been used to induce and study radiation chemistry in numerous systems,[3] including DNA and related molecules. SR has also been used to probe the electronic structure and bonding of such molecules, primarily by probing the occupied states with X-ray photoelectron spectroscopy (XPS) and the unoccupied states with X-ray absorption (XAS) measurements. Bond overlap and localization can be revealed by XPS while XAS can determine the density of unoccupied states and the orientation of the orbitals. In this presentation we examine X-ray induced reactions of DNA adsorbed on a gold substrate when the DNA is either thiolated (tDNA) or when it is unthiolated (uDNA). By performing polarization-dependent XAS at the N K edge we determined that tDNA protrudes from the surface at ~45 degrees, in agreement with previous studies. We also found that the unthiolated molecules have a similar orientation. However, due to differences in charge transfer between the gold and the DNA in the two systems there is a higher density of unoccupied states in the N-C=N derived π^* orbital for tDNA. We also found that the adsorbed tDNA has a significant higher cross section for radiation damage. The reason for this enhancement could arise from the greater probability of forming a TNI state for the tDNA due to the higher density of unoccupied π^* states.

1. E. Alizadeh and L. Sanche, Chem. Rev.**112**, 5578 (2012).

2. R. Naaman and L. Sanche, Chem. Rev.**107**, 1553 (2007).

3. R. A. Rosenberg and S. P. Frigo, in Chemical Applications of Synchrotron Radiation, Part II: X-ray Applications, edited by T.K. Sham (World Scientific Publishing Co., Singapore, 2002), Vol. 12A, p. 462.

2:40pm **BI+AS-TuA2 XPS Binding Energy Shifts for DNA Brushes on Gold**, *C.C.A. Ng*, ***Dmitri Petrovykh***, International Iberian Nanotechnology Laboratory, Portugal

DNA biointerfaces are important in a wide range of existing and emerging applications, such as biosensors, functionalization of nanoparticles for biomedical applications, and self-assembly of complex and functional nanostructures. The complexity of many of the DNA biointerfaces created for such applications often limits the ability to unambiguously interpret the results obtained from spectroscopy measurements for such systems. A powerful and successful approach to improving the analytical capabilities has been based on creating robust and well-defined reference systems, which then provide the insight for data interpretation in more complex analyses. Brushes of oligo(dT) single-stranded DNA can be attached to gold either via terminal thiol linkers, or via terminal blocks of (dA) nucleotides. While the former method results in a brush of roughly upright oligo(dT) strands relatively weakly interacting with one another, the complementarity of (dA) and (dT) blocks within the same strand creates a possibility of intra-strand hairpin-like hybrids in the (dA)-anchored case. Varying the parameters of these DNA brushes and deposition solutions creates a series with expected variation of thickness, surface density, and intra-strand interactions. Gold substrate provides a convenient binding energy (BE) reference for accurate XPS measurements of the characteristic DNA peaks. Following this approach, we find an unexpected BE shift of a N 1s peak across the series of DNA brushes. Typical effects observed in organic films do not appear to account for the full magnitude of the observed shift, so we will discuss the possible interpretations of this effect and its relation to the structure of DNA brushes.

3:00pm **BI+AS-TuA3 Simultaneous 3D Detection of Organics for Intact Samples with Infrared Spectromicrotomography**, ***Carol Hirschmugl***, University of Wisconsin Milwaukee **INVITED**

The holy grail of chemical imaging is to provide spatially and temporally resolved information about heterogeneous samples on relevant scales. Synchrotron-based Fourier Transform infrared imaging1 combines rapid, non-destructive chemical detection with morphology at the micrometer scale, to provide value added results to standard analytical methods. Hyperspectral cubes of (x,y, z, Abs (λ)) are obtained employing spectromicrotomography2, a label free approach, it inherently evaluates a broad array of wide organic materials, with minimal sample preparation and modification. Examples presented here (polymer composites, single cells and colonies of cells) demonstrate the broad applicability of this approach to detect complex chemical information of intact samples.

References

1 Nasse, M. J., Walsh, M. J., Mattson, E. C., Reininger, R., Kajdacsy-Balla, A., Macias, V., Bhargava, R., and Hirschmugl, C. J. (2011) Nat.Methods 8, 413-416

2 Martin, M. C., Dabat-Blondeau, C., Unger, M., Sedlmair, J., Parkinson, D. Y., Bechtel, H. A., Illman, B., Castro, J. M., Keiluweit, M., Buschke, D., Ogle, B., Nasse, M. J., and Hirschmugl, C. J. (2013) Nat.Methods 10, 861-864

Acknowledgements

This work was supported by the US NSF under awards CHE-0832298, CHE-1112433, and DMR-0619759, the Research Growth Initiative of the University of Wisconsin–Milwaukee, and is based on research conducted at the Synchrotron Radiation Center, University of Wisconsin–Madison which is supported by the University of Wisconsin– Milwaukee and University of Wisconsin–Madison.

4:20pm **BI+AS-TuA7 Deep Thoughts: ToF-SIMS Profiling to New Depths**, ***Daniel Graham***, *L.J. Gamble*, University of Washington

The development of argon cluster sources has opened up new opportunities for ToF-SIMS depth profiling. These sources have enabled depth profiling of a wide range of materials that previously could not be accurately depth profiled. In addition, due to the low damage accumulation and sputtering efficiency of these sources, it is now possible to depth profile through microns of material. This in turn has opened up new opportunities for exploring the 3D chemical environments of a wide range of samples including drug eluting polymers, thick multilayer polymer films and porous tissue scaffolds. However, the ability to dig deeper into samples also results in significant challenges in 3D image reconstruction. For example, due to the fixed geometry of the analysis beam (at 45 deg from the surface normal in our instrument), sputtering away 1 micron of the surface will shift the analysis position by 1 micron. This means that if one were to depth profile 50 microns into a surface, the final image would be shifted by 50 microns. Traditional image registrations methods can be used to accommodate for these shifts, however when digging to depths larger than 10 microns, this requires significantly increasing the initial image size in order to end up with a usable image stack after the image shifting and cropping.

In this presentation we will summarize methods we have been developing to reconstruct deep depth profiles including adjusting the sample height during data acquisition and post acquisition image shifting. We will also show results from a new 3D image overlay tool that enables localization of different chemical environments in 3D and that can show areas of overlap between selected peak area images. These methods and tools will be demonstrated on data from control samples made from polymer beads on silicon and from data taken from polymer tissue scaffolds.

4:40pm **BI+AS-TuA8 Development of Novel Pharmaceutical Systems Through Characterisation**, ***David Scurr***, University of Nottingham, UK

The developments in pharmaceutical delivery systems such as injectable drug eluting microparticles [1], topically applied medicines [2] and wound dressings [3] can be utilised in areas such as the treatment of HIV, basal cell carcinoma and microbial infections respectively. In this study, the characterisation of such systems has been performed using time of flight secondary ion mass spectrometry (ToF-SIMS), x-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM).

Injectable controlled release formulations were produced by spray drying two biocompatible polymers, poly(lactic-co-glycolic acid) (PLGA) and polyvinylpyrrolidone (PVP). The samples were analysed using a range of techniques including ToF-SIMS, XPS and AFM showing that the samples were hollow microparticles with a surface PLGA rich phase and an underlying PVP phase [1]. Additionally, more complex ternary systems

incorporating PLGA, PVP and a poorly soluble investigational drug compound were also analysed. These studies highlighted the influence of sample processing parameters and drug concentration upon factors such as surface composition which is influential in the drug release properties of the systems.

The permeation of an antibacterial drug, chlorhexidine, into skin tissue has been illustrated using ToF-SIMS chemical imaging of cross-sectioned treated skin samples [2]. This methodology has been further applied to investigate the topical delivery of imiquimod, a drug used in the treatment of basal cell carcinoma. This work demonstrates the ability of the ToF-SIMS technique to correlate chemical species specific to the drug with physiological features within tissue cross-sections. Further application of ToF-SIMS chemical mapping has also been used to successfully differentiate chemically dissimilar regions of anti-microbial films which could be developed as wound dressing materials. Observations made for these materials using a combination of ToF-SIMS and AFM analysis revealed the distribution of the active agents upon the surface which would be relevant to the the anti-microbial performance.

[1] Meeus, Scurr, Amssoms, Davies, Roberts, and Van den Mooter (2014) Molecular Pharmaceutics, 10 (8)

[2] Judd, Scurr, Heylings, Wan, and Moss (2013) Pharmaceutical Research, 30 (7)

[3] Liakos, Rizzello, Scurr, Pompa, Bayer and Athanassiou (2014) International Journal of Pharmacy, 463 (2)

5:00pm **BI+AS-TuA9 Analysis of Peptide Microarrays on Si Using ToF-SIMS**, ***James A. (Tony) Ohlhausen***, *C. James,* Sandia National Laboratories, *D. Smith,* HealthTell, *S.A. Johnston, N. Woodbury*, Arizona State University

A microarray containing over 1200 each 200µm diameter spots consisting of various length peptide chain monolayers was analysed using Time-of-flight Secondary Ion Mass Spectrometry (ToF-SIMS). This peptide microarray was created using lithographic processes where chains of peptides were built one amino acid at a time. A silane coupling agent was used to attach the peptides to the oxide surface creating a monolayer of peptides directly bonded to the Silicon oxide surface. By tracking ion fragments corresponding to specific amino acids, usually immonium ions, we show that contrast consistent with the number of individual amino acid units in a given peptide dot is generally seen. While some immonium ions are not specific enough to generate clear contrast patterns, most can be used to verify the presence expected amino acids in each peptide dot. Additionally, some amino acids were not found to generate a specific fragment for identification in the positive secondary ion mode.

**Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

5:20pm **BI+AS-TuA10 Investigating Tumor Microenvironments with ToF-SIMS**, ***Lara J. Gamble***, *B. Bluestein, D.J. Graham*, University of Washington

Cancer is a heterogeneous malignancy that manifests itself in a variety of morphological types and clinical outcomes. Current evidence indicates that tumor metabolism plays a large role in cancer onset and progression, and its causes and effects are under intense scrutiny. Furthermore, it is of interest to know where changes in tumor metabolism occur within an affected tissue. However, there are few techniques that can specifically interrogate the tumor microenvironment. We use time-of-flight secondary ion mass spectrometry (ToF-SIMS) to determine differences in the chemical makeup of the tumor microenvironment of breast cancer tumor tissue samples. Human tissue biopsies from an ongoing trial have been subtyped using DASL genome assay and grouped into subtypes of Luminal B, Basal, and ERRB2. Images and spectra have been acquired on an IONTOF TOF.SIMS V using Bi_3^+. The ToF-SIMS information, combined with gene expression array analysis is used to investigate the chemical differences between chemotherapeutic resistant tumors and elucidate the underlying mechanisms. Using imaging ToF-SIMS the cellular and stromal regions within the tissue can be separated out as regions of interest (ROI). Imaging principal component analysis (PCA) was successful in separating cellular regions of the tumor and stromal regions when compared with a hemotoxylin and eosin (H&E) stained adjacent tissue slice. Using the ROIs identified from imaging PCA, we compare the chemical differences between cellular and stromal microenvironment chemistry. A comparison of spectral PCA using the entire analysis area vs spectral PCA of ROIs for cellular and stromal regions of the tissue is discussed. The chemistries of these subtypes are compared using ToF-SIMS image and spectral comparison from cellular and stromal regions. A spectral comparison of ROIs between tissue samples using PCA indicates that unique fatty acids distributions may relate to a tumor phenotype and chemotherapeutic resistance.

5:40pm **BI+AS-TuA11 Correlative Imaging of Mammalian Cells in Their Native Environments using a Microfluidic Reactor by ToF-SIMS and SIM**, ***Xin Hua***, *C. Szymanski, Z.Y. Wang, B.W. Liu, Z. Zhu, J.E. Evans, G. Orr,* Pacific Northwest National Laboratory, *S.Q. Liu,* Southeast University, China, *X.Y. Yu*, Pacific Northwest National Laboratory

Mammalian cell analysis is of significant importance in providing detailed insights into biological system activities. Due to the complexity and heterogeneity of mammalian cell behavior and the technical challenge of spatially mapping chemical components in a hydrated environment, correlated chemical imaging from multiplexed measurement platforms is needed. Fluorescence structured illumination microscope (SIM), with super high resolution and visualization of proteins and sub-cellular structures in 3-D, provides more detailed information in cell imaging. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a unique surface-sensitive analytical tool that provides molecular information and chemical mapping with a sub-micron lateral resolution. However, the understanding of how the spatial heterogeneity and structural difference affect the mammalian cell activities in an unperturbed, hydrated state by ToF-SIMS is severely limited due to the challenge to detect liquids with high volatility under high vacuum environment using surface sensitive technique like ToF-SIMS.

We recently developed a novel microfluidic reactor for C10 mouse lung epithelial cell growth for SIM imaging and direct probing of hydrated cell in vacuum using ToF-SIMS. C10 cells were inoculated into the microchannel, incubated at 37 ^{o}C for 24 hr., fed with 5 nM quantum dots, and then fixed with 4% paraformaldehyde before SIM imaging. In subsequent ToF-SIMS analysis, an aperture of 2 µm in diameter was drilled through SiN membrane to form the detection window to image biological surfaces directly; and surface tension is used for holding the liquid within the aperture.

SIM images show that C10 cells are successfully cultured on the SiN membrane, and quantum dots are uptaken by cells and dispersed in the cytoplasm. The ToF-SIMS *m/z* spectra showing characteristic fragments of dried cell sample, hydrated cells, and uninoculated medium in the microreactor will be presented. Moreover, 2D images of representative cell fragments and quantum dots ion mapping will be discussed. In addition, depth profiling will be used to provide time- and space-resolved imaging of the cells inside the microchannel. Furthermore, principal component analysis is conducted to evaluate the intrinsic similarities and discriminations among samples. Our results demonstrate feasibility for *in situ* imaging of cells in the hydrated state using ToF-SIMS for the first time. Correlative imaging using SIM and ToF-SIMS provides information across different space scales for investigating cell dynamics. This novel approach has great potential for studying intracellular processes in the future.

6:00pm **BI+AS-TuA12 Mass Spectrometry using Femtosecond Lasers and Postionization to Characterize Biomaterials Interfaces**, *Y. Cui, Y.P. Yung,* ***Luke Hanley***, University of Illinois at Chicago

Secondary ion mass spectrometry (MS), matrix assisted laser desorption ionization MS, electrospray-based MS and other strategies are widely used for the analysis of intact bacterial biofilms, mammalian tissue, cell cultures, and their interfaces with biomaterials [Bhardwaj & Hanley, Nat. Prod. Rev. (2014) dx.doi.org/10.1039/C3NP70094A]. The combination of these desorption/ionization methods with high resolution MS and tandem MS capabilities permit metabolomic and proteomic imaging of such samples. Nevertheless, their use to detect many analyte classes within intact biological samples still often suffers from low sensitivity, selective ionization, and/or poor spatial or depth resolution. Laser desorption with ultrashort pulses can remove material from a solid with minimal damage to the remaining sample, potentially allowing both depth profiling and additionally, higher spatial resolution [Cui, *et al.,* ACS Appl. Mater. Interf. 5 (2013) 9269]. Furthermore, laser desorbed neutrals can undergo postionization by vacuum ultraviolet or ultrashort pulse radiation for subsequent detection by MS. Postionization has the additional advantage that proper selection of the delay time between the desorption and postionization laser can improve molecular analysis. Here, we demonstrate the small molecule imaging capability of these methods on intact, multispecies microbial biofilms and other complex organic/biological samples. Finally, comparisons are made to laser desorption MS under atmospheric pressure.

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