Biomaterial Interfaces
Room: 211D - Session BI+AS-MoM

Characterization of Biological and Biomaterials Surfaces (1)

Moderator: Dan Graham, University of Washington, Joe Baio, Oregon State University

8:20am **BI+AS-MoM1 Characterizing the Dissociative Properties of Surface-Bound Biomolecules by *In Vacuo* XPS**, ***Kenan Fears***, Naval Research Laboratory

In vacuo X-ray photoelectron spectroscopy (XPS) was used to determine the dissociation constant for pH-tunable, peptide nanostructures on a gold substrate. To validate these protocols, dissociation constants of GG–X–GG and X_5 peptides (X = G, D, H, or K), and bovine albumin (BSA) and fibronectin (FN) were measured for comparison with published values. Drops of biomolecules in 100 mM sodium phosphate buffers (pH 1-12) were deposited on gold substrates and allowed to dry at room temperature. Due to the ca. +1.3 eV shift in binding energy (BE) of protonated amines, pK values of basic amino acids were calculated by plotting the fraction of protonated amines as a function of solution pH. Similarly, the BE of carboxyl groups shifted ca. -1.3 eV upon deprotonation. While C 1s spectra were convoluted by the multiple chemical states of carbon present in the samples, the ratio of the C 1s components centered at BE=289.0 ± 0.4 and BE=287.9 ± 0.3 proved to reliably assess deprotonation of carboxyl groups. The pK values for the Asp (3.1 & 2.4), His (6.7), and Lys (11.3 & 10.6) peptides, and the pI of BSA (4.8) and FN (5.7), were consistent with published values; thus, validating the pK value obtained for our surface-bound nanostructures using these methods.

9:00am **BI+AS-MoM3 Quantifying the Surface Chemistry and Overlayer Thickness of Functionalized Nanoparticles**, ***David Castner***, University of Washington **INVITED**

Nanoparticles exhibit unique surface properties and require well-controlled surface properties to achieve optimum performance in complex biological or physiological fluids. Despite the widespread appreciation of the unique properties of high surface area nanoparticles there is a surprising lack of detailed surface characterization of these materials, especially for nanoparticles used in biomedical applications. This is in part because nanoparticles present significant challenges for surface characterization. Thus, there is a need to develop rigorous and detailed surface analysis methods for characterizing the surface of nanoparticles. Model systems with well-defined, systematic variations of surface properties are an excellent starting point for developing comprehensive, multi-technique surface characterization methodologies. We have developed methods for quantifying the thickness and structure of carboxylic acid (COOH) SAM functionalized Au nanoparticles (AuNPs) using XPS, SESSA and LEIS. The size, shape, and size distribution of the AuNPs was determined by TEM. Additional surface properties were characterized using ToF-SIMS and FTIR spectroscopy.

These methods were then extended to the covalent attachment of proteins to AuNPs functionalized with OEG SAMs. For the OEG functionalized AuNPs the type of end group (OH vs. OCH_3) doesn't have a significant effect on the SAM thickness and structure, but the size of the AuNP does. The C11 alkyl portion of the thiol molecules was well ordered on all surfaces (flat, 14nm and 40nm). In contrast, the OEG portion of the thiol molecules was better ordered and more densely packed on the 40nm AuNPs compared to the 14nm AuNPs. LEIS measurements showed OEG SAMs had a thickness of 2.0 nm on the 14nm AuNPs compared to 2.6 nm on the 40nm AuNPs. Protein G was immobilized onto the HO-terminated OEG SAMs via carbonyl diimidazole chemistry. On flat Au surfaces XPS showed a monolayer of Protein G was covalently immobilized with little non-specific adsorption. On AuNPs a monolayer of Protein G could also be immobilized, but significant non-specific adsorption was detected.

Recent studies on NPs with Au cores and Ag shells have shown that it is important to account for non-spherical particle shapes of the Ag shell and off-center locations of the Au cores to obtain good agreement between the SESSA and XPS results. Both deviations from ideal core-shell spherical particles result in higher than expected XPS Au concentrations, with the off-center Au cores having the largest contribution to this effect for the particular core-shell NPs examined in this study.

9:40am **BI+AS-MoM5 Structure-Function Relation in Gizzard Plates of Cephalaspidean Gastropod**, *M. Shepelenko, V. Brumfeld, E. Klein,* Weizmann Institute of Science, Israel, *H. Lubinevsky,* Israel Oceanographic & Limnological Research (IOLR) and National Institute of Oceanography, *L. Addadi, S. Weiner,* ***Sidney Cohen***, Weizmann Institute of Science, Israel

Processing food is an essential function of all organisms. Although grinding of food is typically done by teeth, there are a number of species that perform this action in the muscular stomach or gizzard. This places unique demands on the food processing mechanism, a study of which provides fascinating insights into compositional, structural, and mechanical design of the organism at the nanoscale. The Cephalaspidean gastropods are common marine mollusks with a specialized digestive apparatus containing 3 hardened plates of millimeter size inside the gizzard. The gizzard plates are reported to either grind or crush shelled prey. In this study we apply a variety of techniques including micro-CT, scanning electron microscopy with energy dispersive x-ray spectroscopic analysis, infrared and Raman spectroscopies, powder x-ray diffraction and nanoindentation to understanding the manner in which the gizzard plates of the cephalaspid *Philine quadripartita* function in the overall digestion process. We determined that the gizzard plates, used to crush the shelled prey, have distinct structure and composition which promote optimal performance of their function. Specifically, the plate composition, a mixture of amorphous calcium carbonate and amorphous calcium phosphate embedded in a chitinous matrix, varies systematically with depth into the plate. The corresponding elastic moduli and hardness of the plates vary accordingly. In contrast to typical teeth, for which the surface comprises the stiffest and hardest material, the hardest and stiffest layer of the gizzard plates is below the working surface. Analysis of the elasticity index (H/E) of the gizzard plates, and comparison with sea urchin teeth, which we have extensively studied in the past, provided interesting insights into the connection between the biological function and mechanical properties of the gizzard plates. Sea urchin teeth, which serve a grinding function, exhibit higher wear resistance and stiffness than gizzard plates which are used for crushing. Nonetheless, the difference in toughness between the two, as determined by comparison of respective in elasticity indices, is relatively small.

10:00am **BI+AS-MoM6 Photothermal AFM-IR of Bacteria on Polymer Films: Impact of Cantilever Damping on Quantitative IR Measurements**, ***Daniel Barlow***, *J.C. Biffinger,* Naval Research Laboratory, *A.L. Cockrell,* Nova Research, *M. Lo, K. Kjoller, D. Cook,* Anasys Instruments, *W. Kyung Lee, P.E. Pehrsson,* Naval Research Laboratory, *W.J. Goodson,* Air Force Research Laboratory, *J.N. Russell, Jr.,* Naval Research Laboratory

Synthetic polymers can be prone to degradation in microbial and other biological environments, often through enzymatic activity. Quantitative assays are important to characterize these degradation mechanisms and accurately correlate relationships with environmentally dependent microbial physiology. For microbial degradation of polyurethane films, conventional FTIR microscopy has been previously applied in quantitative assays with micron – scale spatial resolution. Photothermal AFM-IR offers the potential to extend this analysis to the nanoscale, allowing early degradation processes and mechanisms relative to single microbes to be quantified. As a first step towards this, we have used AFM-IR to characterize a known polyurethane degrading microbe (*Pseudomonas fluorescens*, Pf01) grown on films of a polyether – polyurethane (PU) formulation known to resist enzymatic degradation. This allowed us to conduct preliminary AFM-IR assessments with a relevant microbe and polymer, but without additional complications from biodegradation. Height images of air-dry samples showed the growth procedure in liquid media resulted in monolayer Pf01 biofilm clusters on top of the ~250 nm PU layer, providing a conducive model system for AFM-IR in an ATR configuration. Both bacteria and PU spectral signatures were detectable by AFM-IR spectroscopy and showed generally good agreement with FTIR. However, PU AFM-IR absorption intensities were observed up to 2x higher in regions covered by dried bacteria, versus uncovered regions, even though the PU thickness was uniform over the substratum. This was due to damping variations which were reflected in the cantilever ring-down and attributed to differences in loss modulus and tip – sample adhesion for the two materials. This shows that local cantilever damping can be an important property to assess in AFM-IR analysis of combined biological / polymer samples, a factor that has received little attention thus far. Analysis of the cantilever ring-down will be discussed regarding extraction of damping parameters for normalization of the IR signal.

10:40am **BI+AS-MoM8 Where's Waldo? 3D Localization of Polymer Nanoparticles in Cells using ToF-SIMS**, ***Daniel Graham***, University of Washington, *J.T. Wilson*, Vanderbilt University, *J. Lai, L.J. Gamble, P.S. Stayton, D.G. Castner*, University of Washington

Polymeric nanoparticles have shown promise for delivery of therapeutics intracellularly. The diversity of polymer chemical and physical properties enables a wide range of cellular targeting and applications. We have initiated a project investigating the use of 3D ToF-SIMS imaging to localize and characterize polymer nanoparticles within cells. Though other imaging modalities can localize polymer nanoparticles in cells, ToF-SIMS presents the advantage of localization combined with chemical characterization of the particles and the surrounding cell. However, the ability to locate polymer nanoparticles in cells is complicated by the fact that most polymers are made of organic elements such as C, N, and O and produce secondary ion fragments that are the same as those generated from the surrounding cell. Herein we will demonstrate a method we have developed to isolate polymer nanoparticle signal from cell signal and generate 3D images of nanoparticle clusters within cells. Initial results with polymer nanoparticles targeted for endosomal uptake showed punctate localization of nanoparticle clusters within areas consistent with endosomal localization. Areas enriched in nanoparticles could be localized in spite of peak overlap of polymer and cellular signals.

11:00am **BI+AS-MoM9 XPS and ToF-SIMS Analysis of Functiionalized Nanoparticles: Effects of Sample Cleaning and Preparations**, *R. La Spina, V. Spampinato, I. Ojea, F.J. Rossi, D. Gilliland,* ***Giacomo Ceccone***, European Commission, Joint Research Centre, IHCP, Italy

It is recognized that detailed physico-chemical characterization of nanomaterials is becoming increasingly important both from the technological and from health and safety point of view. Moreover, an incomplete characterisation may inhibit or delay the scientific and technological impact of nanoscience and nanotechnology. However, nanomaterials characterization based on individual instrumental methods is a very challenging issue because their stability, coating and environmental effects may lead to outputs that are not very easy to interpret unequivocally. For this reason multiple analysis methods are needed to understand the nature of nanomaterials, especially if we consider that surface and interfaces are critical to the behaviours of nano-sized materials [1].

Surface chemical analysis methods, such as X-ray Photoelectron Spectroscopy (XPS) and Time of Flight Secondary Ion Mass Spectrometry, can provide an important contribution to more fully characterizing nanomaterials, so these methods should be more generally applied as part of a characterisation set of tools for nanomaterials and nanoparticles synthetized for different applications [2].

In this work, we have investigated the surface chemistry of nanoparticles, gold (AuNPS) and silica (SiO2NPs), functionalized with different thiols. In particular, the effects of sample cleaning by centrifugation and dialysis have been studied. Moreover, the challenges and problems related to sample preparation for the surface analysis will be also addressed and discussed The different steps of sample cleaning have been characterised by DLS, CPS and SEM, whilst the surface chemistry has been mainly assessed by XPS. Our results indicate that the cleaning process may influence the functionalization process. For instance, the AuNPs functionalized with CF3 terminated SAMs shows differences in the efficiency depending upon sample cleaning.

Finally, preliminary results about the behaviour of AuNPs-CF3 in protein solution (HSA) will be also presented.

[1] Baer D, et al., Anal. Bioanal. Chem., **2010**, 396(3), 983–1002

[2] Grainger D and Castner D, Adv. Mater., **2008**, 20, 867–877

11:20am **BI+AS-MoM10 Engineered Surfaces for Bio-Relevant Applications**, ***Marlon Walker***, NIST, *A. Vaish*, University of Delaware, *D. Vanderah*, NIST/Institute for Bioscience and Biotechnology Research

Polydopamine (PDA) is a useful bio-inspired coating for surface modification. Substrates from noble metals such as gold to semiconductors such as silicon can be modified to exhibit useful biomimetic properties that may not be available on the underlying surface. However, conditions of preparation can lead to wide variability in the attributes (such as roughness) of the generated surface, and can affect subsequent functionalization and applicability. Wider adoption of the routine use of PDA is hindered by this uncertainty of the nature of the prepared surface. We present strategies for greater control of the properties of a PDA coating, which could lead to enhanced predictability of surface attributes and greater utility in surface engineering strategies.

11:40am **BI+AS-MoM11 Breast Cancer Tumor Metabolism Investigated with ToF-SIMS**, ***Lara Gamble***, *B.M. Bluestein, D.J. Graham*, University of Washington

Imaging time-of-flight secondary ion mass spectrometry (ToF-SIMS) was utilized to analyze 21 breast tissue biopsy samples. Eighteen of the biopsy samples were obtained at diagnosis and three after neoadjuvant therapy. Principal component analysis (PCA) was used to reduce the spectral data and determine major variants in the data. PCA analysis of the mass spectral data was used to test for correlation to phenotypes (ER+/PR+, HER2+, and ER-/PR-/HER2-) as well as determine the chemical changes pre and post neoadjuvant therapy.

PCA imaging analysis of the ToF-SIMS tumor tissue images showed that the combination of PCA and ToF-SIMS imaging was able to distinguish different tissue regions that correspond with similar regions in H&E stained serial tissue slices from the same block. Most notably the stromal and cellular regions could be distinguished by imaging PCA. Utilizing regions of interest (ROIs), chemical makeup of stromal regions from different tumor biopsy samples was compared.

While the cellular region showed the clearest separation for pre and post treatment chemistry, spectral PCA analysis of the stromal region shows better separation in scores plots when comparing different tumor types. Chemical analysis of the stromal regions also separated out chemical differences in triple negative tumor samples (with five different triple negative rated tumors investigated to date). In an initial sample set, the pCR (patient complete recovery) and ‘near’ pCR samples both score negatively in the PC2 scores plot. The key fatty acids associated with pCR samples are myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0) and a 20:3 fatty acid as well as fragments of sphingomyelin and various triglycerides. The main peaks associated with the non-pCR samples were fatty acid 18:1 (consistent with oleic acid) along with cholesterol and vitamin E related peaks. Coincidentally these peaks correlate well with the loadings from the pre neoadjuvant therapy treatment samples, while the highest loadings from the pCR samples correlate with the post treatment tissue loadings.

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