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

Characterization of Biological and Biomaterials Surfaces (2)

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

2:20pm **BI+AS-MoA1 Characterization of Protein G B1 Immobilized Gold Nanoparticles using Time of Flight Secondary Ion Mass Spectrometry and X-ray Photoelectron Spectroscopy**, ***Yung-Chen Wang****, *D.G. Castner*, University of Washington

Nanoparticles (NPs) have been widely used in many fields of science due to their unique physical properties. While many applications of NPs such as imaging probes or drug carriers often require the conjugation of proteins or biomolecules, the surface interactions between NPs and biomolecules remains underexplored. For example, the immobilization of immunoglobulin G (IgG) onto NP surfaces is critical for the development of many immunosensors and drug delivery nanocarriers. Notably, the orientation of the immobilized IgG can have a significant impact on clinical outcomes of nanocarriers by impacting its biostability and efficacy. One approach to control the proper orientation of IgG is by utilizing the IgG Fc tail binding proteins.

In this work, Protein G B1, a protein that will selectively bind to the Fc tail of IgG, was immobilized onto gold NPs (AuNPs) functionalized with maleimide and oligo-(ethylene glycol)(OEG) self-assembled monolayers (SAMs). Protein G B1 was immobilized on AuNPs using either carbonyldiimidazole (CDI) chemistry or maleimide-cysteine interaction. We use the surface sensitive analysis techniques of x-ray photoelectron spectroscopy (XPS) and time of flight-secondary ion mass spectrometry (ToF-SIMS) to characterize the immobilization of protein G B1. Unlike conventional NP characterization techniques such as dynamic light scattering (DLS) and UV/Vis, XPS and ToF-SIMS can provide additional information on the surface elemental composition, protein coverage and orientation.

XPS analysis confirmed the CDI activation of the OEG-SAMs AuNPs by detecting the nitrogen containing active intermediate and the attenuation of gold signal. After incubation with protein, the immobilization of the protein was demonstrated by the increased nitrogen signal on the surface. ToF-SIMS analysis also confirmed the successful functionalization, CDI activation, and protein immobilization by identifying signature secondary ions from each step of the protein immobilization process.

By comparing the ratio of secondary ion intensity originating from opposite ends of the protein, it was possible to determine the orientation of immobilized protein G B1. As expected, the non-site specific CDI chemistry did not lead to a specific protein orientation on the AuNPs. In contrast to CDI chemistry, we expect to control the orientation of the immobilized protein using maleimide functionalized AuNPs and cysteine mutants of Protein G B1 through site-specific carbon-sulfur interaction.

Overall, the systematic characterizations in this study will provide detail information of protein-NP interactions and serve as a platform for controlling the orientation of IgG on AuNPs.

2:40pm **BI+AS-MoA2 Controlled Molecular Mechanisms of Engineered Solid Binding Proteins on Surfaces**, ***Christopher So***, National Research Council postdoc cited at Naval Research Laboratory, *S. Walper*, US Naval Research Laboratory, *R. Stine*, Nova Research, *D.E. Barlow*, *K. Wahl*, US Naval Research Laboratory

Persistent and uncontrolled aggregation of proteins at surfaces remains a major challenge for biocompatibility, fouling, and biosensing. To fully realize the rich properties of proteins at interfaces, a critical link between displayed protein sequence and surface assembly mechanisms is required. Here we use rational protein mutations combined with *in situ* microscopy and spectroscopy methods to demonstrate that manipulation of solid binding and intermolecular interactions by proteins can dictate their surface behavior and induce nanostructure formation. We use streptavidin (SA) as a robust scaffold to control the density and localization of aromatic residues, expected to interact with surfaces such as graphite and graphene through pi-bonding. The surface adapted SAs are generated by placing aromatic side chains of varying polarity (Phenylalanine, Tyrosine, Tryptophan) along three putative permissive sites in a coplanar arrangement. The effects of these mutations on bulk solution structure, surface-associated structure, as well as surface affinity, orientation and spatial organization are studied *in situ* using attenuated total reflectance (ATR) infrared spectroscopy (IR) with linear polarization (LP), fluid-mode atomic force microscopy (AFM), and circular dichroism (CD). We have found that our simple modifications to mSA have little effect on the solution state of the protein, while having a pronounced effect on affinity and secondary structure in the adsorbed state. Through fabricating graphene-coated ATR-IR prisms, we find that unmodified mSA exhibits an ordered beta sheet structure at surfaces, while tryptophan modifications to mSA (Trp-mSA) induces a more disordered structure. We quantify by temporal ATR-IR spectra a *ca.* 4.5x enhancement in sticking probability for Trp-mSA over mSA to graphene. Fluid-mode AFM studies on graphite support a surface-mediated coarsening mechanism: while mSA forms no obvious surface structures, Trp-mSA aggregates and forms islands 10-50 nm in size over the course of an hour. Such disordered SA aggregates provide high affinity sites for slow lateral island growth processes, giving rise to a bi-modal exponential adsorption curve for Trp-mSA but absent in mSA. Ultimately, defining the molecular basis of protein self-assembly and the impact of displayed chemistries at liquid-solid interfaces will enable rationally designed biological surface coatings and engineered biointerfaces with tailorable functionalities.

3:00pm **BI+AS-MoA3 Molecular-Level Surface Analysis Demonstrates the Impact of Detergent Selection on Decellularized Tissues**, ***Adam Taylor***, University of Washington, *L.J. White*, University of Nottingham, UK, *D.M. Faulk*, *L.T. Saldin*, University of Pittsburgh, *D.G. Castner*, University of Washington, *S.F. Badylak*, University of Pittsburgh, *B.D. Ratner*, University of Washington

Decellularized matrix scaffolds may be prepared through a range of techniques. Detergents are frequently used in decellularization protocols due to their ability to solubilize cell membranes and dissociate DNA from proteins. Whilst removal of cellular material is regularly assessed, the impact of detergent selection on extracellular matrix (ECM) structure and composition is less commonly investigated. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a powerful surface analysis technique to probe biological structures with high mass resolution and surface specificity, and has previously been used to distinguish decellularized ECM by anatomical location or culture conditions. The objective of this study was to utilize ToF-SIMS to investigate the influence of detergent selection upon a representative decellularized tissue, specifically the basement membrane complex (BMC) of porcine urinary bladder matrix (UBM) prepared by treatment with 1% SDS, 4% deoxycholate, 8 mM CHAPS or 3% Triton X-100 for 24 hours.

Principal components analysis (PCA) revealed spectral differences between treatment groups. High mass peaks associated with specific detergent fragments were observed on the scaffolds exposed to SDS and deoxycholate. Peaks indicative of phospholipid membranes were observed in all samples, but to a greater extent with scaffolds not exposed to detergent. We further probed these data sets to investigate how detergent selection impacts proteinaceous ECM components. Using a reduced peak list of known characteristic amino acid fragments, PCA distinguished native bladder tissue from decellularized UBM and highlighted spectral differences between UBM treated with ionic vs. charge-neutral detergents. Notably, the basement membrane surface of UBM prepared with ionic detergents SDS and deoxycholate yielded less intense characteristic peaks from hydrophobic amino acids than UBM treated with charge neutral detergents CHAPS and Triton X-100. Harsher detergents may denature protein structure and break protein-protein interactions through binding of their hydrophobic tail to hydrophobic amino acid residues. Such damage is hypothesized to cause sub-optimal in vitro and in vivo responses. We further examined cell-matrix interactions of human urothelial cells seeded on the BMC of UBM, investigating how detergent exposure affected cell proliferation and permeability of the cell monolayer. An understanding of the effects of detergent exposure on the structure, composition and surface molecular functionality of decellularized scaffolds will facilitate a rational strategy for successful recellularization and subsequent positive clinical outcomes.

3:20pm **BI+AS-MoA4 Liquid Repelling Surfaces Based on Candle Soot are Non-Fouling**, ***Lars Schmüser***, *M. Paven*, *N. Encinas*, Max Planck Institute for Polymer Research, Mainz, Germany, *D.J. Graham*, *D.G. Castner*, University of Washington, *D. Vollmer*, *H.J. Butt*, *T. Weidner*, Max Planck Institute for Polymer Research, Mainz, Germany

Super non-fouling surfaces resist protein adhesion and have a broad field of possible application like implant technology, drug delivery, blood compatible materials, biosensors and marine coatings. Non fouling properties can be fabricated by using liquid repelling surfaces, which

*** ASSD Student Award Finalist**

minimize the contact area of water soluble particles with the non fouling surface. For a surface to be "amphiphobic" – to repel a range of liquids including oil and water – requires a micro to nanometer scale surface roughness in combination with a hydrophobic coating. Paven et al. (*1*) described the production of an amphiphobic surface with remarkably low production requirements. This surface is made of a glass slide, candle soot and 2 commercially available chemicals which are deposited via chemical vapor deposition. Soot deposition and chemical vapor deposition can be applied to a broad variety of substrate shapes, such as the inner wall of tubes. This makes the soot coating a promising tool for blood compatible material design for stents and tubing including applications such as dialysis. Here we present a protein adsorption study onto these amphiphobic surfaces made of candle soot. Since even nanograms per cm^2 levels of protein on biomaterial surfaces can cause detrimental effects for patients, we employed surface sensitive spectroscopic methods, X-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectrometry (ToF-SIMS) to quantify protein adsorption. We did not detect any adsorbed proteins within a detection limit of better than 1 ng/cm² of adsorbed proteins, which demonstrates the super non-fouling property of soot-coated surfaces. Interestingly, the naturally amphiphobic cuticle ("skin") of springtails – small ancient arthropods who live in soil – use an approach very similar to the artificial soot surfaces to achieve protein repellency: Nanometer roughness with hydrophobic coatings. We will discuss XPS, ToF-SIMS and fluorescence microscopy studies quantifying the amount of protein adsorbed onto these surfaces.

1. M. Paven *et al.*, Super liquid-repellent gas membranes for carbon dioxide capture and heart–lung machines. *Nat Commun***4**, (2013).

3:40pm **BI+AS-MoA5 Time-of-Flight Secondary Ion Mass Spectrometry Investigations of the Pancreatic Islet Tumor Microenvironment**, ***Blake Bluestein***, Department of Bioengineering, University of Washington, *F.M. Morrish, D. Hockenbery*, Fred Hutchinson Cancer Research Center, *L.J. Gamble*, Department of Bioengineering, University of Washington

Imaging time-of-flight secondary ion mass spectrometry (ToF-SIMS) provides chemical information with subcellular spatial resolution. In this work, imaging ToF-SIMS is used to analyze tumor microenvironments from mouse model (Myc/p53-/-) biopsies with Myc-dependent inducible and regressible pancreatic β-cell neoplasia. The Myc oncogene is overexpressed in many human cancers and has major effects on cellular metabolism, including lipid metabolism. While imaging ToF-SIMS analysis of tumor tissue will provide a new perspective by visualizing tumor progression/regression, the system itself can also act as a model system for investigating stroma-tumor interactions in cancerous tissues.

Pancreatic tissues were harvested and frozen in optimal cutting temperature (OCT) at 6 days post Myc induction. 4 µm cryosections were serially cut, with one used for H&E staining, one for ToF-SIMS analysis, and another for immunohistochemistry. High mass and high spatial resolution data was acquired with the pulsed 25 keV Bi_3^+ ion beam rastered over a 1 mm x 1 mm area (1280 x 1280 pixels). ROIs of the tumor and stromal tissue were then investigated further with imaging principal components analysis (PCA) to identify peaks that correspond to species of interest. Regions identified by analysis and PCA were cross-referenced against immunohistochemical and H&E images to differentiate tumor areas from the surrounding tissue.

ToF-SIMS data suggests a preferential uptake of fatty acids 18:3 and 18:2 within the tumor. The 6 day Myc-induced islet tumor exhibits a signal of 14:0, possibly a product of de novo fatty acid synthesis within the tumor. The tumor also exhibits an increased localization of sphingomyelin fragments and vitamin E compared to the surrounding tissue. Interestingly, the data shows an absence of Mg^+ within the islet tumor and small, higher signal regions on the periphery of the tumor. These peripheral tumor regions also show an increased, localized signal of CN^-, CNO^-, $C_7H_{10}O^+$, and Fe^+, but further histologic correlations are needed to discern if these structures are inflammatory zones, mitochondrial dense regions, or related to vasculature. Once these localized areas have been defined, a comparison to the chemistry identified by ToF-SIMS may aid in interpreting the Myc oncogene and its effect on pancreatic β-cell neoplasia. PCA was applied to image data and revealed different chemistries within the tumor and surrounding tissue. PCA was also applied to selected tumor region images to spatially and chemically analyze within the tumor to compare chemistries between different tumor sizes, where tumor size is potentially indicative of different tumor stage development.

4:00pm **BI+AS-MoA6 Paper-based Device for Home Phenylalanine Monitoring from a Sample of Whole Blood**, *R. Robinson*, ***Elain Fu***, Oregon State University

Paper microfluidics is a rapidly growing subfield of microfluidics that makes use of paper-like porous materials to create devices for use in low-resource settings. Advantages of the use of porous materials include capillary flow, removing the need for equipment for pumping fluids, and lower material costs compared to traditional microfluidics-based devices composed of silicon or glass. In the current presentation, we describe the development of a paper-based device for home therapy monitoring. For persons with phenylketonuria (PKU), maintaining a restricted level of phenylalanine (Phe) in the body is a continuing challenge. Given the large inter-person variation in Phe metabolism, maintaining nutritional therapy can be a lengthy and difficult process that would be aided by the ability to perform real-time monitoring of Phe levels. Adherence to diet therapy is an even greater challenge for young children, adolescents, and women during pregnancy, and for these groups in particular, rapid feedback could be critical in tailoring a diet to be optimal for each individual. Current tests for Phe require a high-resource laboratory environment and are not suitable for the rapid detection of Phe levels and feedback to the patient that is needed for effective monitoring of PKU therapy. Our solution is a semi-quantitative, paper-based device that is rapid, easy to use, and low cost for patient home use. Device operation is based on simple user steps. The user applies whole blood (40 mL) to a plasma separation membrane, which filters out the cellular components of the blood and releases plasma to two downstream glass fiber pads. There, Phe in the sample and NAD+, catalyzed by the enzyme phenylalanine dehydrogenase, react to form Phe-pyruvate, NADH, and NH_3. At 6 min, the user folds the card closed and fluid is transferred to a final glass fiber detection pad, in which NADH, nitroblue tetrazolium, and methoxy phenazine methosulfate react to form NAD+ and a purple-colored product. The device is read at ~7.5 min. Visibly distinct signal intensities are generated from whole blood samples containing 0 (normal), 3.75 (slightly elevated), and >7.5 mg/dL (substantially elevated) spiked-in Phe. Thus, this test may allow users to distinguish between normal versus elevated levels of blood Phe on a rapid timescale that could inform their diet therapy. The assay exhibited reasonable reproducibility with coefficients of variation between 11 and 24%. A focus of the presentation will be on the controled patterning and drying of biochemical reagents in porous materials for later rehydration on the device, which is key to the robust operation of the device.

4:20pm **BI+AS-MoA7 Multivalent Probes for Tuneable 'Superselective' Targeting**, *G.V. Dubacheva*, CIC biomaGUNE, Spain, *T. Curk*, University of Cambridge, UK, *R. Auzély-Velty*, Cermav, Cnrs, France, *D. Frenkel*, University of Cambridge, UK, ***Ralf Richter***, CIC biomaGUNE & University Grenoble Alpes, Spain **INVITED**

A basic requirement in biomedical research is the ability to specifically target cells and tissues. Targeting typically relies on the specific binding of a 'ligand' on a tailor-made probe to a 'receptor' on the desired cell or tissue. Conventional probes efficiently distinguish a cell surface displaying the receptor from others that do not. They exhibit limited selectivity, however, when the surfaces to be distinguished display a given receptor at different densities.

Based on theoretical arguments, it has been proposed that multivalent probes that bind several receptors simultaneously can sharply discriminate between different receptor densities. Here, we present an experimental model system that demonstrates such 'superselective' targeting. To this end, recent achievements of synthetic chemistry and surface characterization were combined to create well-defined multivalent polymers and surfaces that interact with each other through highly specific host/guest interactions. With this model system, we show that superselective binding can be tuned through the design of the multivalent probe to target a desired density of binding sites. We develop an analytical model that provides simple yet quantitative predictions to tune the polymer's superselective binding properties by its molecular characteristics such as size, valency, and affinity.

This work opens up a route toward the rational design of multivalent probes with defined superselective targeting properties for practical applications in life sciences (analytics, diagnostics and therapy). It also provides mechanistic insight into the regulation of multivalent interactions in biology, notably the superselective targeting of the extracellular matrix polysaccharide hyaluronan to its main cell surface receptor CD44.

5:00pm **BI+AS-MoA9 Targeted Ultrathin Silica Nanoshells as HIFU Sensitizing Agents for *In Vivo* LnCAP Prostate Tumor Removal**, ***James Wang***, *A. Liberman, C. Barback, S. Blair, R. Mattrey, W. Trogler, A.C. Kummel*, UC San Diego

Diagnostic ultrasound (US) is a prevalent medical imaging modality due to its low-cost, high resolution, and therapeutic capability when coupled with high intensity focused ultrasound (HIFU) systems. 500 nm rigid silica ultrathin nanoshells were synthesized as a chemically stable US tumor marking contrast agent with continuous *in vivo* US imaging lifetime. Iron (III) was included into the silica shell network to promote biodegradability from serum transferrin proteins. It was shown previously that the removal of iron from the silica shell network via transferrin fragments the nanoshells for effective biodegradation. Folate was conjugated to the surface of the

silica nanoshells via the 3-aminopropyltriethoxysilane (APTES) linker. Folate has been shown in the literature to bind to prostate specific membrane antigen (PSMA) with a high binding affinity due to folate hydrolase activity. Conjugating the silica nanoshell surface with folate targets the ultrathin silica nanoshells towards the LnCAP tumor where PSMA is significantly up-regulated. The surface modified ultrathin silica nanoshells were filled with liquid perfluorocarbon (PFC) which underwent acoustic droplet vaporization (ADV) during US insonation. The phase transition of PFC from liquid to vapor generated a large amount of PFC microbubbles that created contrast during US imaging. *In vitro* experiments with US have demonstrated that the ultrathin silica nanoshells can be imaged for at least 3 hours under color Doppler imaging, exhibiting a continuous US imaging lifetime. *In vivo* experiments have shown that folate conjugated silica nanoshells were able to accumulate and persist within the tumor region for up to 12 days post-injection, observable with US imaging. Surface conjugation with polyethylene glycol (PEG) increased the ultrasound signal at the tumor by increasing the particles accumulating at the tumor site. When exposed to high intensity focused ultrasound (HIFU), the particles were able to enhance the HIFU power and liquefy tumor tissue. With particles present, the HIFU duty cycle can be lowered to 2 %, minimizing tissue thermal deposition. By synthesizing ultrathin silica nanoshells with a folate-conjugated surface, it is has been demonstrated that folate-conjugated ultrathin rigid silica nanoshells can accumulate in the LnCAP tumor persistently for 12 days. PEGylation of the particles further increase the particle accumulation concentration in the tumor, acting as a HIFU sensitizing agent for ultrasound histotripsy. Through intelligent surface modification, liquid PFC filled silica particles can act as a multi-functional theranostic agent for ultrasound diagnosis.

5:20pm **BI+AS-MoA10 Transparent Field Effect Sensor with Nanostructured Amorphous In-Ga-Zn-O Wires**, ***Xiaosong Du***, *Y. Li*, *J. Motley*, *G. Herman*, Oregon State University

Amorphous In-Ga-Zn-O (a-IGZO) materials have a wide range of applications in high performance electronic devices, from the active material in thin film transistors for flat-panel displays and as the transducer for field effect sensors. A key benefit of a-IGZO over amorphous silicon is that it enables low processing temperatures, while retaining relatively large electron mobilities, low operating voltages, and very low off currents. In this study, we have fabricated a-IGZO films with well-defined nanostructures using colloidal lithography. These nanostructured a-IGZO films were then patterned into wires using electrohydrodynamic printing of an etch resist followed by wet chemical etching. We have characterized these nanostructured a-IGZO wires using field effect test structures to evaluate their electronic properties. To improve selectivity and stability of the nanostructured a-IGZO wires for sensing applications we have functionalized the back-channel surface with molecular receptors, where glucose oxidase was successfully attached as a sensing enzyme. Depletion/accumulation of carriers in the a-IGZO back-channel was observed upon reaction of the glucose oxidase with the analyte, which leads to significant changes in the sensors electronic signals. Continuous monitoring of glucose concentration can be achieved by measuring a direct change in channel conductance, turn on voltage shift, and/or electrical hysteresis. The results obtained for nanostructured a-IGZO wires will be compared to blanket a-IGZO films, where we have found that the nanostructured a-IGZO wires provide a significant enhancement in sensitivity to subtle changes in glucose concentrations in physiological buffers. These results provide insight into a route to develop low-cost transparent biochemical sensors based on the emerging a-IGZO technology.

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