Thursday Afternoon, October 31, 2013

Biomaterial Interfaces
Room: 102 B - Session BI+AS+BA+NS+SS-ThA

Biomolecules at Interfaces
Moderator: S.M. McArthur, Swinburne University of Technology, Australia

2:00pm **BI+AS+BA+NS+SS-ThA1 The Protein Resistance Properties of Hydroxy- and Methoxy-terminated Oligo(ethylene oxide) (OEO) Self-Assembled Monolayers (SAMs) to Membrane Proteins**, ***M. Walker***, National Institute of Standards and Technology (NIST), *A. Vaish, D. Vanderah*, National Institute of Standards and Technology (NIST) and Institute of Bioscience and Biotechnology Research

Spectroscopic ellipsometry was used to evaluate the resistance to protein adsorption of self-assembled monolayers (SAMs) of $HS(CH_2)_3O(CH_2CH_2O)_6M$ and $[HS(CH_2)_3]_2CHO\text{-}(CH_2CH_2O)_6M$, where M = CH_3 or H, on Au. The SAMs were exposed to fibrinogen, a soluble protein frequently used to evaluate surface protein resistance properties, and rhodopsin, an integral membrane protein. We show that the that the nature of the oligo(ethylene oxide) end group governs the extent of protein adsorption resistance of OEO SAMS to integral membrane proteins.

2:20pm **BI+AS+BA+NS+SS-ThA2 A Bottom-up Approach for Creating Biomimetic Surfaces with Defined Nanotopographic Structure and Surface Chemistry**, ***N.P. Reynolds***, *K.E. Styan, C. Easton,* CSIRO Materials Science & Engineering, Australia, *R. Mezzenga,* ETH Zürich, Switzerland, *B. Muir, P. Hartley*, CSIRO Materials Science & Engineering, Australia

Interactions of tissue cells with their local microenvironment (the extracellular matrix) can be split into three distinct categories: 1) Physical interactions, such as cellular responses to elasticity or stiffness, 2) chemical interactions, with specific epitopes contained within the extracellular matrix, and 3) topographical interactions with the nanoscale fibrous proteins that make up the majority of the extracellular matrix. In order to study how these interactions affect cell physiology *in vitro,* biomimetic substrates can be designed to reproduce these interactions. Whilst there have been multiple examples of substrates that accurately mimic chemical and physical interactions, the effects of truly biomimetic topographies are less well explored.

We show for the first time it is possible to use networks of self-assembled amyloid fibers as templates for the deposition of plasma polymers under high vacuum conditions. The nanoscale topography of the underlying amyloid networks is replicated on the top surface of the polymers with remarkable fidelity, resulting in a chemically homogenous surface with well-defined nanoscale surface features that mimic the topography of the extracellular matrix. The culture of fibroblast cells on these substrates resulted in an increased cell attachment and spreading compared to flat polymer films. We show evidence that the increase in favorable cell spreading was caused by a stabilization of adsorbed serum proteins (including fibronectin) by the nano-topography. Thus, we hypothesize that the reduced denaturation of proteins on the nano-topographical substrates results in matrix adhesion moieties (e.g. the RGD sequence) being presented to the cell membrane in a more physiological orientation. This templating technique allows for the rapid and reproducible fabrication of substrates with nanoscale biomimetic topography. We believe that such surfaces will have applications in the development of new biomaterials that will allow the routine investigation of physiological nanoscale morphology on cellular phenotype.

N.P. Reynolds et. al., "Nano-topographic surfaces with defined surface chemistries from amyloid fibril networks can control cell attachment" Biomacromolecules, **2013**, DOI: 10.1021/bm400430t.

2:40pm **BI+AS+BA+NS+SS-ThA3 Nanoscale Imaging of Peptide-Membrane Interactions**, ***P.D. Rakowska***, National Physical Laboratory, UK

Antimicrobial peptides (AMPs) are attracting growing attention as efficient anti-infective agents in the post-antibiotic era. However, the detailed molecular mechanisms of their action and precise rationale for their selectivity remain poorly understood.

Here we will present our recent findings, highlighting specific membrane-mediated mechanisms of AMPs, which we probed using a de novo designed archetypal AMP and imaged using a combination of Atomic Force Microscopy (AFM) and high-resolution Secondary Ion Mass Spectrometry (NanoSIMS). This approach provides unique information on the topography of peptide-treated membranes, obtained from AFM images, suggesting membrane changes as a result of peptide structuring and pore formation. The data is complemented by chemical imaging performed on the same samples with NanoSIMS, which revealed the precise localization of peptide molecules in the membranes.

This comparative topographical and chemical imaging gives the first evidence of antimicrobial pore expansion that was further strengthened by AFM imaging in real time in liquid, and supported by microbiological and biophysical studies as well as molecular dynamic simulations.

Relevant publication:

Rakowska, P. D., Jiang, H., Ray, S., *et al*. Nanoscale imaging reveals laterally expanding antimicrobial pores in lipid bilayers. *Proc. Natl. Acad. Sci. USA,* **2013**, 110, in press.

3:00pm **BI+AS+BA+NS+SS-ThA4 Development of Molecular Modeling Capabilities in LAMMPS Specifically Designed for the Efficient and Accurate Simulation of Biomolecule-Surface Interactions**, ***R.A. Latour***, Clemson University, *C.D. Lorenz*, King's College-London, UK

The ability to understand and predict the interactions of tethered or adsorbed biomolecules (e.g., protein, DNA, carbohydrates) at material interfaces represents a critical need for many applications in bionanotechnology and biomedical engineering. Experimental methods alone are typically very limited in terms of their ability to probe the molecular level of detail needed to quantitatively understand these types of complex interfacial interactions. As a result, biomaterial systems must often largely be designed by trial-and-error approaches. Molecular simulation methods provide an excellent means to complement experimental studies to provide theoretical assessment and predictive capability of the behavior of biomolecules at interfaces with atomic-scale resolution. These methods, however, must be specifically designed and developed for biomaterial applications. The Latour group has focused on the development of molecular simulation methods for the efficient and accurate simulation of protein-surface interactions over the past two decades, mostly involving the CHARMM molecular simulation program. Over the past year, we have focused on transitioning from CHARMM to the LAMMPS molecular simulation program for our continued development work in collaboration with the Lorenz group at King's College-London. LAMMPS (Large-scale Atomic/Molecular Massively Parallel Simulator) is a fast, versatile, and highly parallelizable molecular simulation program with excellent capabilities for materials modeling. It is also freely available for download from the primary developer's website (Sandia National Laboratory, <http://lammps.sandia.gov/>). Over the past year, we have been developing new LAMMPS modules that are specifically being designed to support the efficient and accurate simulation of protein-surface interactions, with planned extension to other biomolecule systems. In this presentation, we will provide an overview of the developed capabilities in the LAMMPS program, with demonstrated applications to simulate protein-surface interactions at the atomic level. The development of these molecular simulation modules in LAMMPS has the potential to revolutionize current capabilities to accurately simulate, predict, and understand mechanisms governing biomolecule interactions at material interfaces and to serve as a valuable tool for system design.

3:40pm **BI+AS+BA+NS+SS-ThA6 Dynamic Nanomaterials for Diagnostics and Drug Delivery**, ***P. Stayton***, University of Washington **INVITED**

Our group develops stimuli-responsive nanomaterials that utilize dynamic structural and architectural transitions to enable new drug delivery and diagnostic functionalities. For drug delivery applications we are focused on opening the intracellular target universe to biologic drugs. Biologic drugs such as DNA, RNA and proteins have significant therapeutic potential, but effectively formulating and delivering them remains a widely recognized challenge. Barriers include drug stability, tissue penetration and transport, but cytoplasmic entry is a widespread barrier for those that function against intracellular disease targets. We have been developing synthetic polymeric carriers that mimic the highly efficient intracellular delivery systems found in pathogenic viruses and organisms. Another important aspect of these polymeric carriers is the development of controlled polymerization techniques to streamline bioconjugation of targeting agents and therapeutics, as well as to generate controlled carrier architectures. The carriers might open up new families of peptide, antibody or nucleic acid drug candidates that attack previously inaccessible intracellular targets. For diagnostic applications we are addressing the technology gap for making clinical assays faster and more sensitive, as well as the need for simple yet efficient sample handling techniques that concentrate dilute biomarkers for

point-of-care (POC) tests. We have developed a new stimuli-responsive magnetic nanoparticle reagent system for achieving both of these goals. These new bioanalytical systems are being applied to clinical lab assays, lab card disposable devices and for non-instrumented lateral flow diagnostic platforms.

4:20pm **BI+AS+BA+NS+SS-ThA8 Determination of Orientation and Tertiary Structure of Adsorbed Protein on Material Surfaces by Chemical Modification and Peptide Mapping**, ***A.A. Thyparambil***, *Y. Wei, R.A. Latour*, Clemson University

Chemical modification of targeted amino acid residues with peptide mapping via mass spectrometry (MS) is a promising technique to provide highly detailed information on the structural shifts and orientation of adsorbed protein by revealing adsorption-induced changes in amino acid solvent accessibility. A decrease in amino acid labeling (i.e., decreased solvent accessibility) is indicative of adsorbed orientation while an increase is indicative of tertiary unfolding. However, the potential of this method for the study of adsorbed protein structure is largely undeveloped at this time. The objective of our research was therefore to develop chemical modification and peptide mapping techniques that would help identify the dominant configuration of adsorbed protein on a material surface for a range of amino acid types. By directly comparing the extent of amino acid modification (profiles) from separate batch experiments targeting different types of amino acids, a fairly detailed picture of adsorption-induced changes in adsorbed protein structure can be obtained. In our current study, when unmodified segments of the protein without the targeted amino acid from the MS results was used as an internal standard for each of the batch experiments, a common baseline to directly compare the profiles of different amino acids could be obtained. Under these conditions, the configuration of hen egg white lysozyme (HEWL) when adsorbed on fused silica glass (glass), high density polyethylene (HDPE), and poly(methyl–methacrylate) (PMMA) was mapped by directly comparing the profiles of arginine (Arg), lysine (Lys), tryptophan (Trp), and carboxylic groups (Asp, Glu, C-terminus). For each of the targeted amino acid groups, the labeling procedure did not induce significant structural shifts, which was verified by circular dichroism spectropolarimetry. The resulting quantitative differences in the profiles of targeted amino acid residues in HEWL on different surfaces under different conditions correspond to different configuration of HEWL on each adsorbent surface. The developed technique has the potential for broad application and to be expanded to other targeted amino acids, thus providing highly detailed information on the adsorbed state of protein on any given surface.

4:40pm **BI+AS+BA+NS+SS-ThA9 Exploring the Formation, Lifetime and Dissociation Statistics of Acid-Amine Bonds**, *S. Raman*, ***M. Valtiner***, Max Planck Institute für Eisenforschung GmbH, Germany

Acid-amine interactions are non-covalent, long-range interactions, contributing to the structural integrity in manmade adhesives and to serve complex life functions in several biological systems. Understanding how these interactions develop and alter over time in an aqueous environment, especially when presented across an interface, is vital when it comes to designing functional surfaces for biomedical applications. We use single molecule force spectroscopy to investigate the contact dynamics of molecular bonds under near-physiological conditions. We explore the interactions of NH_2/COOH bonds that are presented across the atomic force microscopy (AFM) tip-surface interface, with much focus on the dissociation of these bonds by studying specific signatures obtained during the force measurements[#]. Since the approach permits us to have an exquisite of control over the interface, a number of experimental parameters are varied such as the number density of the molecules, ionic strength of the surrounding medium and extension/retract speed of the tip to vary the loading rate. A statistical evaluation of the interactions and contact dynamics is discussed to assess the influence of the experimental parameters on the bond dissociation. The transition rate under zero-load conditions is calculated combining the detachment statistics and Kramer Evans theory. Our results provide new insights into the binding regime and dissociation behavior of acid-amine bonds from non-equilibrium to near-equilibrium conditions as a function of the loading rate on a logarithmic scale in aqueous environments of varying ionic concentration.

[#] M Valtiner, SH Donaldson , MA Gebbie, JN Israelachvili, J. Am. Chem. Soc., 2012, 134, pp 1746–1753.

5:00pm **BI+AS+BA+NS+SS-ThA10 Thiolene Reaction Applied to Different Metal Oxide Surfaces: Role of Short and Long PEG-terminated Chains on Biomolecules Solution Adsorption**, ***A. Galtayries***, *A. Dellinger*, Chimie ParisTech, France, *V. Semetey*, Institut Curie, France

The control of biomolecules adsorption (such as proteins) and other microorganisms is of high interest for various fields of biotechnology, such as bioanalytics, cell biology, tissue engineering and biomaterials. A simple and efficient method to control adsorption includes the use of the thiolene chemistry to form self-assembled monolayer (SAM) from commercial long (poly(ethylene glycol)) and short (oligo-ethylene glycol) terminated chains, applied on metal oxide surfaces [1].

Both on silicon, titanium and iron-chromium substrates, we selected two polymers either with short or long chains: one is adhesive, the other one is non-adhesive once in interaction with solutions of biomolecules. As regards short-chain molecules, the adhesive O-(2-Mercaptoethyl)-O′-methyl-hexa(ethylene glycol) and the adhesive O-(2-Carboxyethyl)-O′-(2-mercaptoethyl) heptaethylene glycol further activated by reaction with N-hydroxysuccinimide (NHS) were selected for grafting strategies implying full surface grafting or adhesive/non adhesive patternings (100 micrometer-large bands or half-moon surfaces). Similarly, as long-chain molecules, poly(ethylene glycol) methyl ether with an average molecular weight of 5,000 have been used, adhesive ones being NH_2-terminated.

With such molecular selection, we performed a systematic study using surface characterization techniques such as X-ray Photoelectron Spectroscopy (XPS), Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) and Infra-Red Surface Spectroscopy (ATR-IRFT or PM-IRRAS): at different steps of the grafting process, as well as after interaction with protein solutions, surface qualitative as well as quantitative information where obtained to discuss the efficiency of these molecular strategies to build biointerfaces on metal oxide surfaces.

[1] ``A Facile and Versatile Approach to Design Self-Assembled Monolayers on Glass using Thiol-ene Chemistry", B. Oberleitner, A. Dellinger, M. Déforet, A. Galtayries, A.-S. Castanet,V. Semetey, Chemical Communication, 49, 1615-1617 (2013).

5:20pm **BI+AS+BA+NS+SS-ThA11 Immobilization of Peptide-Based Stimuli-Responsive Biomolecules on Silica Surfaces**, ***L. Li***, *O. Im, J. Harris, W. Han, A. Chilkoti, G.P. López*, Duke University

The immobilization of stimuli-responsive biomacromolecules onto silica surfaces is often performed the development of silica-based biosensors, protein microarrays and supramolecular assemblies. The R5 silaffin peptide, derived from *Cylindrotheca fusiform*, is of current interest because of its capacity to induce and regulate silica precipitation at ambient conditions. In this study, we found that a fusion protein comprised of a synthetic silaffin R5 peptide and elastin-like polypeptide (ELP) bound reliably to silica particles and flat silica-based surfaces. ELPs are a class of stimuli-responsive polypeptides that undergo a reversible lower critical solution temperature (LCST) phase transition. In silaffin-ELP fusion proteins, the R5 peptides serve as silica-binding domains that immobilize ELPs onto silica, allowing its surface properties to be modulated upon change in temperature through the LCSTs of the ELPs. The attachment of silaffin-ELP to silica particles was confirmed by temperature- and time-dependent turbidity, zeta potential, and dynamic light scattering measurements. As demonstrated through zeta potential measurements, the positively charged silaffin-ELPs neutralized the negative charge on the silica particles, confirming the binding of silaffin-ELPs. Dynamic light scattering experiments revealed an increase in particle size after surface modification. The sizes of surface-modified particles also changed in response to temperature. We also investigated the absorption of silaffin-ELP on oxidized silicon wafers. The elemental composition of the protein-modified surfaces was characterized by X-ray photoelectron spectroscopy. We also used ellipsometry and atomic force microscopy (AFM) to test the thickness and roughness of the protein bound surfaces. Contact angle measurements were performed to examine the temperature-responsive nature of the surfaces. Furthermore, we demonstrated that GFP-ELP fusion protein can be adsorbed to silaffin-ELP modified silica surface through co-aggregation above their LCSTs. A thermally triggered aggregation behavior of fluorescently-labeled silica particles was also visualized using confocal fluorescence microscopy. The results of this study demonstrated that a silaffin tag can be used to immobilize ELPs on silica surfaces such as silica particles, silicon wafers and glass slides, and that these protein-modified surfaces can be used to capture and immobilize ELPs and ELP-fusion proteins reversibly onto their surfaces. This system has potential uses in bioseparations, biomaterials, and biosensors.

5:40pm **BI+AS+BA+NS+SS-ThA12 Microfluidic Extraction and Labeling of Methylated DNA from Small Cell Populations for Single-Molecule Analysis**, ***J. Benitez***, *J. Topolancik, H. Tian, C. Wallin, V. Adiga, P. Murphy, J. Hagarman, P. Soloway, H.G. Craighead*, Cornell University

We describe a microfluidic device for the extraction, labeling, and purification of human chromosomal DNA from single cells and small cell populations. The extracted and labeled material was quantified using single-molecule fluorescence analysis in nanofluidic channels. A two-dimensional array of micropillars in a microfluidic polydimethylsiloxane (PDMS) channel was designed to capture cells. Megabase-long DNA strands released from the cell upon lysis are trapped in the micropillar array and

stretched under optimal hydrodynamic flow conditions. Chromosomal DNA is immobilized in the array, while other cellular components are washed away from the channel. To assess DNA methylation, genomic DNA from different cell types was extracted using the device and labeled on-chip with methyl-CpG binding domain 1 (MBD1) protein. MBD1-bound DNA was released from the device and directly transferred to a nanofluidic channel for single-molecule detection of MBD1 molecules. Individual DNA fragments and MBD1 proteins were driven electrophoretically through the nanofluidic channels. The photon counts obtained from each MBD1 detection event are directly proportional to the total number of MBD1 molecules. By quantifying the amount of bound MBD1 molecules, the DNA methylation abundance of each cell type can be assessed and compared. This methodology provides a means for epigenetic fluorescence analysis of small cell populations with single-molecule resolution, extendable to single cells.

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