## Biomaterial Surfaces & Interfaces
## Room Milo - Session BI-MoM

### Buddy Ratner's 70th Birthday Session
**Moderator:** Lara Gamble, University of Washington, USA

8:00am **BI-MoM1 SIMS Surface Science from SAMs to 6S Scaffolds***, **Daniel Graham**, L.J. Gamble,* University of Washington, USA

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) generates chemically rich, complex data that can encode information about surface composition, molecular conformation, orientation and more. However, due to the complexity and magnitude of the data, it is imperative that research projects be carefully planned out before the researchers attempt to extract this information using ToF-SIMS. Professor Ratner understood this challenge and recognized that, after utilizing a well planned research strategy, applying multivariate analysis methods (MVA) could aid to better understand ToF-SIMS data and use it more efficiently. This idea, generated more than 20 years ago, led me on a journey exploring the complexities of ToF-SIMS through the use of self-assembled monolayers (SAMs) that has continued into exploring complex organic systems such as cells, tissues and tissue engineered scaffolds. In this presentation I will highlight the work pioneered by the ideas of Buddy Ratner that helped start the MVA revolution in SIMS analysis and has led to the development of methods that help to more efficiently process and better understand secondary ion mass spectrometry data. Examples will be shown from controlled experiments with SAMs, cells, tissues and tissue engineering scaffolds. Work with SAMs helped demonstrate that combining MVA with SIMS and well controlled substrates could help us better understand the SIMS fragmentation process and discover new information encoded in the relative intensities of the peaks. This set the foundation for processing more complex systems and using MVA and SIMS to characterize the chemical differences in more complex systems. This has led to one of our current projects involving characterizing tissue engineering scaffolds with cells in 2D and 3D.

8:20am **BI-MoM2 Genetic Level Programming of Molecular Assembly of Intrinsically Disordered Proteins***, **Gabriel López**,* University of New Mexico, NSF Research Triangle Materials Research Science and Engineering Center, Duke University, USA **INVITED**

A number of dynamic, protein-rich intracellular structures containing phase separated, unstructured proteins comprising low-complexity amino acid sequences have recently been shown to serve a variety of important cellular functions, including signaling, compartmentalization and stabilization. The understanding of these structures, and the ability to synthesize models of them, has been limited. This talk will present simple methods for programming diverse assemblies comprised of a series of elastin-like polypeptides, model intrinsically disordered proteins possessing sequences of low-complexity. By encoding the stimulus-induced aqueous phase behavior of proteins at the amino acid sequence level, we demonstrate the reversible formation of a variety of protein-rich structures, ranging from uniform nano-, meso-, and micro-scale puncta (small, distinct particulates) to multilayered, orthogonally-phase-separated, multicomponent microgranules. We further show how such nanoscale assemblies (i) can be stabilized by controlled biomineralization, (ii) can be used for simple bioassays for diagnostic or drug discovery applications, or (iii) can be used as building blocks for the hierarchical formation of micellar hydrogels with surprising mechanical properties and potential use in controlled delivery of nanoparticles for drug delivery applications. The talk is dedicated to Prof. Buddy Ratner, a mentor and friend of mine and of my collaborator, Prof Ashutosh Chilkoti, on the occasion of Buddy's birthday this year.

9:00am **BI-MoM4 Surface Activation of the VWF A1 Domain: The Relationship between Platelet Activity and Absorbed A1 Structure***, H. Tronic, E. Thomas, **David Castner**,* University of Washington, USA

When a material is placed in a biological environment, the surface of the material acts as the interface between that material and the biological environment. Upon contacting blood, plasma proteins attach to these surfaces and mediate platelet adhesion and activation and thrombosis. A key protein in this process is the clotting protein von Willebrand Factor (VWF) which binds to platelet receptor glycoprotein 1bα (GPIbα) when VWF is activated by chemicals, high shear stress, or immobilization onto surfaces. Activation of VWF by surface immobilization is an important problem in the failure of cardiovascular implants, but is poorly understood. Here we investigate whether some or all surfaces can activate VWF at least in part by affecting the orientation or conformation of the immobilized GPIbα-binding A1 domain of VWF. Platelets translocate rapidly on A1 adsorbed onto PS surfaces, and demonstrate shear-enhanced adhesion in that they detach at low rather than high shear stress. In contrast, platelets translocate more slowly on A1 adsorbed onto TCPS surfaces and are nearly stationary on A1 adsorbed onto glass surfaces, and demonstrate shear-inhibited adhesion in that they detach at high but not low shear stress. Both X-ray photoelectron spectroscopy and conformation independent antibodies reported comparable A1 amounts on all surfaces. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) suggested differences in orientation on the three surfaces, but none that could explain the biological data. Instead, ToF-SIMS determined Cys exposure and conformation-sensitive antibody binding suggest that A1 retains its native conformation when adsorbed onto PS surfaces, while TCPS surfaces and especially glass surfaces stabilized an alternative activated conformation of A1 that likely resembles the activated form of A1 that is also stabilized by disease-causing mutations. Regardless of the specific structure of the activated forms of A1, these studies demonstrate that it is not enough to determine the amount of various proteins that bind to different biomaterials placed in contact with the blood; instead, it is necessary to understand how different surfaces control the conformation of the many blood proteins that are capable of undergoing activating conformational changes. In particular, these studies demonstrate that the A1 domain of VWF has fundamentally different biological activity when adsorbed onto different surfaces. This is important when interpreting or designing *in vitro* experiments with surface-adsorbed A1 domain, and is also of likely relevance for blood-contacting biomaterials.

9:20am **BI-MoM5 Why do Bacteria Stick to Some Surfaces and Not Others? Characterisation of the Behaviour of Motile Bacteria at and above the Surface of Materials***, **Morgan Alexander**,* University of Nottingham, UK, United Kingdom of Great Britain and Northern Ireland

Antimicrobial resistance has been recognised as a pressing problem by the WHO, the UK government review [Jim O'Neill 2014] predicting a financial impact equal to cancer by 2050 and most recently a unanimous declaration by the UN General Assembly. Infections associated with medial devices are a significant contribution to this challenge. Hook et al. used high throughput screening to discover a new class of polymer with resistance to biofilm formation correlating with the chemistry of the uppermost nanometer of the material. [Nature Biotechnology 2012] Whilst a device using this material is progressing to regulatory approval for use in man, we are exploring the mechanism by which these work to enable us to develop improved devices.

Microorganisms cannot be approximated to inert objects since they possess surface responsive appendages such as flagella, which enable them to swim, pili that confer twitching motility and fimbriae that mediate surface attachment in response to surfaces. These 'devices' are in turn coupled to sophisticated signal transduction mechanisms that facilitate integration of multiple local environmental parameters at both single cell and population levels. Many of these sensory systems are postulated to contribute to surface sensing. As an example of the complexity of these processes, the opportunistic pathogen Pseudomonas aeruginosa has over 60 two-component sensor kinase response regulator systems involved in environmental adaptation.

We believe that bacterial decision-making is key to determining whether a surface is colonised or not. I will present the early results from our optical microscopy investigations of how individual bacterial cells respond to surfaces. We have developed a novel microscope that collects temporal 3D information on cell position using both holography and remote scanning microscopy. Surface tracking can be simultaneously achieved using DIC, TIRF and TIR microscopy. This allows us to track not only the motion of single cells at the surface, but also their approach to and behaviour after contact with the surface. We will combine these findings with our understanding of the surface chemistry-attachment relationships for certain subsets of materials and attachment regimes with in situ chemical analysis to build a complete description of this complex biointerface and the response of bacteria to it. This information is crucial in determining how bacteria behave with respect to defined surfaces and has important implications for the prevention of device centred infections and the development of the next generation of biofilm resistant surfaces.

9:40am **BI-MoM6 Antibody Microarrays for Point-of-Care Detection from a Single Drop of Blood***, **Ashutosh Chilkoti**,* Duke University, USA

I will discuss a point-of-care diagnostic that we have developed, in which all reagents are printed and stored on a "non-fouling"—protein and cell resistant—polymer brush. The D4 assay, involves four sequential events:

(1) Dispense (droplet of blood); (2) Dissolve (printed reagents on chip); (3) Diffuse (across surface); and (4) Detect (binding event). The D4 POCT antibody (Ab) consists of microarrays printed on the polymer polymer brush yields quantitative results, with picomolar sensitivity within 30 minutes. All reagents are inkjet-printed and stored on D4 POCT cassettes, which do not require refrigeration. Upon direct application of fingerstick blood onto a cassette, analyte capture and detection occur automatically, generating a quantifiable fluorescence signal obtained by placing the cassette in a small device that magnetically attaches to a smart phone, which images and analyzes microarrays via on-board App. Examples of quantitative dose-response from whole blood will be presented. The D4 assay can be used for the diagnosis of all markers for which antibody pairs are available with a speed and sensitivity that is as good or better than commercially available point-of-care tests and is far simpler, cheaper more rugged, and does not require a cold-chain.

10:20am **BI-MoM8 Plasmas, Proteins and Other Things Buddy has Inspired me to Play with in Vacuum Chambers**, ***Sally L. McArthur***, Swinburne University, Australia **INVITED**

Control and the ability to elicit specific responses from a biological system lies at the heart of most bioengineering. We want to immobilize proteins on biosensors but ask them to behave as they would in the body, stimulate cells to assemble into tissues, reconstructing our bodily functions. We want methods that prevent bacteria forming biofilms and better still we would like them to stop bacteria attaching to surfaces full stop. But biology is soft and normally has lots of water associated with it, so how and why would you want to use vacuum based techniques to create coatings or characterise these systems? This talk will explore how in my group and our collaborators, have tackled the challenges associated with interfacing vacuum deposited plasma polymers with water, proteins, lipids and cells to create a wide number of model systems and devices. At the same time, we have developed methods for chemically characterising these systems in vacuum, integrating XPS and ToF-SIMS with a range of other surface analytical and biological tools to gain insight into the materials we create and their interactions with biological systems.

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