Biomolecules at Aqueous Interfaces Focus Topic
Room: 203 A - Session BA+AI+AS+BI+IS+NL-MoM

Biomolecules at Aqueous Interfaces
Moderator: P. Koelsch, University of Washington

8:20am **BA+AI+AS+BI+IS+NL-MoM1 Selected Studies of Biomolecular Interactions**, ***K.B. Eisenthal***, *B. Doughty, Y. Rao, S.M. Kazer, S.J.J. Kwok, N.T. Turro*, Columbia University **INVITED**

The work reported here utilizes a sensitive method for the investigation of biomolecular interactions that has the important characteristic of not requiring chemical labels, e.g. fluorophores, nor invasive detection methods. The surface selective second order spectroscopies, second harmonic, SHG, and sum frequency generation, SFG, allow one to probe equilibrium properties and time dependent changes in the electronic and vibrational structure of molecules located at interfaces. In addition they have the special feature of being able to monitor changes in the electrical charge of the interacting molecules. 1) A new way is presented to measure the binding constants of molecules, e.g. drugs and proteins, with DNA tethered to colloidal microparticles suspended in aqueous solution. 2) Time resolved second harmonic generation was used to observe the binding of an enzyme to its recognition site on DNA, followed by the cleaving of DNA into a small and a large fragment, and the subsequent DNA rehybridization dynamics. 3) The relative orientation of two molecules bound to DNA is manipulated by changing the number of nucleotide base pairs separating them. The interference between the SH electric fields generated by the pair of molecules is modulated because their relative orientation changes as the number of nucleotide base pairs separating them is changed. With this method we have a new way to probe structural changes in DNA due to the binding **of** biomolecules to it.

9:00am **BA+AI+AS+BI+IS+NL-MoM3 Probing Nanoparticle-lipid Bilayer Interactions with Nonlinear Optics**, ***F. Geiger***, Northwestern University **INVITED**

The interaction of engineered nanoparticles with biological membranes is an important and necessary first step for cellular uptake. Here, we probe this interaction by applying second harmonic and vibrational sum frequency generation as well as the Eisenthal chi(3) method to supported bilayer-based model systems as well as shewanella and daphnia magna, chosen as important biological endpoints, exposed to 4 nm sized noble metal nanoparticles surrounded by negatively and positively charged ligands. Our studies are complemented by a plethora of supporting experiments based on quartz crystal microbalance, zeta potential, and related experiments. We find that Coulomb's law dictates much of the interactions in the particular systems studied here.

9:40am **BA+AI+AS+BI+IS+NL-MoM5 Characterizing the Protein-Surface Interactions that Control Diatom Biomineralization**, ***J.E. Baio***, Oregon State University, *M. Bonn, T. Weidner*, Max Planck Institute for Polymer Research, Germany

The assembly of mineralized tissues can be initiated and controlled by proteins. One such system, is the formation of silica-based cell walls in marine, single celled organisms, where biomineralization is regulated by protein−mineral interactions. The diatom species *Cylindrotheca fusiformis* assembles supramolecular silica structures via proteins called sillafins. In a silicic acid solution, specific repeat units within this protein, SSKKSGSYSGSKGSKRRIL (R5), induce the formation of silica-protein composite nanoparticles. The protein−surface interaction that drives self-assembly is likely controlled by both the secondary structural motifs of the protein and specific contacts between the surface atoms and key protein side chains. In this study, we characterized the R5-SiO_2 interactions that drive this self-assembly process by both near edge x-ray absorption fine structure (NEXAFS) spectroscopy and *in situ* sum frequency generation (SFG) spectroscopy. Two peaks within the amide I vibrational band of the SFG spectra, 1640 and 1670 cm^{-1}, indicate that the R5 peptide retains a beta sheet conformation when interacting with SiO_2. Expanding upon this characterization of secondary structure, the introduction of isotopic labeled amino acids within the peptide allowed us to probe the orientations of individual side chains by SFG. This SFG characterization was complemented by the observed polarization dependence of the NEXAFS C1s to π^* transition which provided details of the binding geometry of the single tyrosine within R5.

10:00am **BA+AI+AS+BI+IS+NL-MoM6 Probing the Effects of Different Ions on the Formation of Microstructure Within Collagen Hydrogels by Second Harmonic Generation (SHG) Microscopy**, ***X. Lang***, *J.G. Lyubovitsky*, University of California, Riverside

In this study we aimed to explore the nucleation, assembly and the 3-D microstructure of collagen hydrogels *in situ* with second harmonic generation (SHG) microscopy. Transmission electron microscopy (TEM) and optical density (OD) were carried out as well in order to complement the SHG measurements. The goal was to generate the knowledge to accelerate rational design of collagen-based biomedical products. In this work, we employed 0, 150, 300, 600, 900 mM NaCl concentrations and in a separate experiment 0, 5, 10, 20, 50, 75, 100, 150, 300 mM Na_2SO_4 concentrations of salts needed for the assembly of collagen hydrogels. Specifically, we characterized collagen hydrogels prepared from 2 g/l and 4 g/l initial collagen concentrations as well as several incubation temperatures. For samples incubated with NaCl, incubation under the room temperature (RT) and 27°C gave similar OD values. These OD values were higher than the OD values for 37°C incubated samples. Delay time became shorter upon elevating the polymerization temperature. For samples incubated with Na_2SO_4, there were two regimes for collagen polymerization, Na_2SO_4 concentration 5 mM – 50 mM and 100 mM -300 mM. Fibers were longer when NaCl concentration was 150-600 mM compared to 0 and 900 mM NaCl for both collagen concentrations under RT, 27°C and 37°C. In general, fibers were small when incubated at 37°C compared to fibers formed under RT and 27°C. TEM measurement showed that there were collagen fibers with a characteristic striation structure in all collagen and NaCl concentrations. The fibrils exhibited a twisted morphology in 2 g/l collagen hydrogels.

10:40am **BA+AI+AS+BI+IS+NL-MoM8 Sum Frequency Generation (SFG) Vibrational Spectroscopy Studies of Molecules at Solid-Liquid and Solid-Gas Interfaces**, ***G.A. Somorjai***, University of California, Berkeley and Lawrence Berkeley National Laboratory, *X. Cai*, Lawrence Berkeley National Laboratory **INVITED**

Construction of a femtosecond broad-band laser enables us to simultaneously monitor the CH and CO vibrational SFG spectra, thus allowing more actual characterization of reaction intermediates at solid-liquid and solid-gas interfaces. Using a picosecond laser we compare the spectroscopy using these two different laser systems and they will be discussed in some detail. In addition, sum frequency generation vibrational spectroscopy, high-pressure scanning tunneling microscopy and ambient-pressure X-ray photoelectron spectroscopy as well as other synchrotron-based techniques (X-ray adsorption) that enable the investigation of surfaces under reaction conditions on the atomic and molecular level will be reviewed.

We investigate solid-liquid and solid-solid interfaces (buried interfaces) as they adsorb and react with diatomic and organic molecules in dynamic state at various pressures and temperatures.

11:20am **BA+AI+AS+BI+IS+NL-MoM10 A Molecular View of Water Interacting with Climate-active Ice Nucleating Proteins**, *R. Pandey*, Max Planck Institute for Polymer Research, Germany, *J. Fröhlich, U. Pöschl*, Max Planck Institute for Chemistry, Germany, *M. Bonn*, ***T. Weidner***, Max Planck Institute for Polymer Research, Germany

Specific bacteria, such as *Pseudomonas syringae*, effectively attack plants by using ice-nucleating proteins anchored to their outer cell surfaces. Ice nucleating proteins promote the local crystallization of ice at temperatures that would otherwise not allow ice formation. The frost damage caused by ice crystals then facilitates bacterial invasion of the affected plants. Ice nucleating proteins not only play an important role for agriculture, but are also very important for atmospheric processes: airborne ice-nucleating proteins have been shown to be among the most effective promoters of ice particle formation in the atmosphere. A recent survey of microorganisms in the troposphere biome by NASA has discovered massive emissions of biogenic ice nucleators from large forest areas like the amazon, which likely change precipitation patterns and may affect the global climate. To understand biogenic ice formation, a detailed molecular level picture of the mechanism by which ice-nucleating proteins interact with water molecules is important. Sum frequency generation (SFG) spectroscopy – owing to its inherent interface sensitivity – is ideally suited to determine the structure and dynamics of water molecules at interfaces. We have investigated the interaction a monolayer of the ice-nucleating protein inaZ with water using static and time-resolved SFG spectroscopy. When cooling the sample from room temperature to near-freezing temperatures (~5°C for D_2O), inaZ significantly increases the structural order of water molecules in contact with inaZ proteins. This effect was not observed for liquid water surfaces

without the protein or for protein monolayers which are not ice nucleators. SFG spectra in the CH and the amide I region also indicated a change of protein structure near the nucleation temperature. Femtosecond, time-resolved 2-dimensional SFG spectroscopy is used to quantify the heterogeneity of protein-bound water molecules and their structural dynamics.

Biomaterial Interfaces
Room: 201 B - Session BI+AI+BA+IS-MoA

Biofouling
Moderator: D.E. Barlow, Naval Research Laboratory

2:00pm **BI+AI+BA+IS-MoA1 Biofouling of Carbon Steel: Effects of Microstructure and Test Media on Initial Bacterial Attachment and Subsequent Corrosion**, *M.A. Javed, P.R. Stoddart, S.M. McArthur,* ***S.A. Wade***, Swinburne University of Technology, Australia

Biofouling of surfaces causes numerous problems in a wide range of industries such as shipping, health care, oil and gas production and food production. Of specific interest to the current work is the accelerated corrosion of metals that can arise as a consequence of bacterial biofilm formation, which is commonly known as microbiologically influenced corrosion (MIC).

The initial attachment of bacteria to a surface is one of the first steps in the process of biofouling. The attachment is dependent upon a large number of factors, which are broadly related to the properties of the bacteria, substrate/surface and environment. Changes in these properties can not only influence the initial attachment step, but also the interrelated production of extracellular polymeric substances (EPS) by the bacteria and the subsequent corrosion.

A large amount of the work performed to date on bacterial attachment in relation to MIC has focused on stainless steels, possibly due to reports of rapid failures of these materials such as through thickness pitting of piping welds. These studies have highlighted how a range of material properties (e.g. chemical composition, surface roughness, grain size and boundaries) can influence attachment and biofilm formation on steel surfaces. This range of influences means that a high level of care must be taken when designing and carrying out bacterial attachment tests in order to avoid the situation where a number of material variables affect the outcome of a single test. For example one of the criticisms of some of the previous work in this area is the lack of control of surface roughness of the substrates used in the studies.

In this work we will report results of studies of the initial attachment and EPS production of *E. coli* bacteria on highly polished carbon steel samples, with a number of different microstructures, for a number of different test media. We have found that the microstructure and test medium can have a significant effect on the rate of bacterial attachment, the distribution of attached bacteria, the onset of EPS production and the corrosion of samples immersed in *E. coli* inoculated test media.

2:20pm **BI+AI+BA+IS-MoA2 Charged SAMs as Model Surfaces to Understand Anti-fouling Properties of Zwitterionic Coatings**, ***S. Bauer***, University of Heidelberg, Germany, *J. Finlay, M.E. Callow, J.A. Callow,* University of Birmingham, UK, *A. Rosenhahn*, Ruhr-University Bochum, Germany

Zwitterionic surfaces are a class of coatings that receive increasing attention due to their good antifouling performance.[1] Since early work on protein resistance of mixed, charged self-assembled monolayers (SAMs), charge neutrality seems to be a prerequisite for their inert properties. [2,3] Similar to established non-fouling ethylene glycol chemistries, zwitterionic systems rely on a strong hydration of the coating. In this study we attempt a systematic analysis to which extend charge neutrality and the chemical nature of the charged groups affect their antifouling performance. Positively charged trimethylammonium terminated thiols were therefore mixed with sulfonate-, carboxylate- and phosphonate-terminated undecanethiols in varying ratios. Optimized preparation conditions and surface analysis will be presented that demonstrates successful assembly of the coatings and characterizes their physicochemical properties. The antifouling properties were tested against a range of laboratory organisms such as diatoms and spores of algae and compared to protein resistance. The obtained trends will be discussed and correlated with field experiments in the real marine environment.

(1) Chen, S.; Jiang, S. **2008** A new avenue to nonfouling materials. *Advanced Materials*, 20, 335-338.

(2) Holmlin, R. E.; Chen, X. X.; Chapman, R. G.; Takayama, S.; Whitesides, G. M. **2001** Zwitterionic SAMs that resist nonspecific adsorption of protein from aqueous buffer. *Langmuir*, 17, 2841-2850.

(3) Chen, S. F.; Yu, F. C.; Yu, Q. M.; He, Y.; Jiang, S. Y. **2006** Strong resistance of a thin crystalline layer of balanced charged groups to protein adsorption. *Langmuir*, 22, 8186-8191.

2:40pm **BI+AI+BA+IS-MoA3 The Role of Bacterial Physiology in Biodeterioration of Polyurethane Coatings**, *S. Zingarelli,* Air Force Research Laboratory, *D.E. Barlow, J.C. Biffinger,* Naval Research Laboratory, *L.J. Nadeau,* Air Force Research Laboratory, *D. Babson,* Naval Research Laboratory, *B.W. Stamps,* University of Oklahoma, *R.K. Pirlo,* Naval Research Laboratory, *C.N. Drake,* Air Force Research Laboratory, *B.S. Stevenson,* University of Oklahoma, *J.N. Russell, Jr.,* Naval Research Laboratory, ***W.J. Crookes-Goodson***, Air Force Research Laboratory **INVITED**

Microbial biofilms frequently contaminate surfaces and can cause degradation of polyurethane coatings that are intended to protect against environmental degradation. Historically, investigations of polyurethane biodeterioration have focused on identification and characterization of the organisms and 'polyurethanase' enzymes involved in the degradation process. However, many questions remain unanswered. For example, microbes capable of polymer degradation are ubiquitous in the environment, yet only affect polymers under some circumstances. What controls the production of polyurethanases? What is the role of planktonic vs. biofilm populations in the biodeterioration process? The goal of our research is to define the parameters and regulatory mechanisms that result in polyurethane biodeterioration by *Pseudomonas protegens Pf*-5, with a focus on environmental conditions (nutrients, pH, oxygen) and microbial 'lifestyles' (planktonic vs. biofilm populations). First, we screened a variety of carbon sources with a polyurethane agar plate-clearing assay using the polyester polyurethane Impranil DLN. Results showed that strain *Pf*-5 could grow on a variety of carbon sources but that degradation of polyurethane varied depending on the carbon source. We observed strong polyurethane degradation in the presence of M9-citrate medium but severely reduced clearing of polyurethane when glucose was provided as a carbon source. Subsequent studies with planktonic cultures of *P. protegens Pf*-5 verified the inhibitory effect of glucose on polyurethanase activity. Using proteomic tools, activity in citrate-grown planktonic culture supernatants was ascribed to two esterases, polyurethane esterases A and B. Currently the regulation of these enzymes is being investigated through a combination of genetic and transcriptomic approaches. Biofilms were grown on Impranil DLN in M9-citrate or -glucose to determine if these nutrients also regulated polyurethanase secretion in biofilms. Micro ATR-FTIR surface chemical analysis of the coatings after biofilm removal showed that degradation proceeds through preferential loss of the ester component. However, optical microscopy and profilometry clearly show that subsequent bulk coating loss can occur under certain conditions, resulting in complete loss of the original coating surface, and eventually complete loss of the coating. Transmission FTIR microscopy was also used to detect bulk coating degradation in a biofilm culture plate assay we developed to complement the Impranil clearing assay. This assay demonstrated significant Impranil coating degradation from citrate-grown biofilms versus minor degradation for glucose-grown biofilms.

3:40pm **BI+AI+BA+IS-MoA6 Multifunctional Active Nano and Microstructured Surfaces for Biofouling Management**, ***G.P. López***, Duke University **INVITED**

This talk will present (i) recent developments of stimuli responsive surfaces that exhibit dynamic structure on lateral length scales of the order of 10 microns and below, (ii) a prospectus for the formation of multifunctional bioactive surfaces based on such dynamic micro- and nanostructured materials, and (iii) results from study of bioadhesion and biorecognition on these surfaces. Stimuli responsive polymer surfaces include patterned polymer brushes and elastomers; biological systems of interest include protein solutions, adherent mammalian cell lines, as well as marine and infectious bacteria. Our previous studies have demonstrated that stimuli responsive polymers can be used to control the adhesion of such systems and, in this presentation, we will provide our latest advancements in this line of study, as regards to both molecular and cellular biointerfacial phenomena. Methods for preparing dynamic micro- and nanopatterns of stimuli responsive polymers will be presented, along with characterization of their structure, dynamic behavior and bioadhesion resistant character.

4:40pm **BI+AI+BA+IS-MoA9 Roles of Extracellular DNA in the Development and Expansion of Bacterial Biofilms**, ***C.B. Whitchurch***, University of Technology, Sydney, Australia **INVITED**

Biofilms are multicellular communities of bacteria that are often found attached to surfaces and cause significant problems in medical, industrial, and marine settings. Cells within biofilms are enmeshed in an extracellular polymeric matrix comprised of polysaccharides, proteins, lipids, and nucleic acids. Over the past decade, extracellular DNA (eDNA) has been found to be essential for biofilm formation by many species of bacteriawhere it is

thought to function as an intercellular “glue” that binds cells together. Interestingly, whilst it has been known for over a decade that eDNA is essential during the early stages of biofilm development by the opportunistic pathogen *Pseudomonas aeruginosa*, the precise roles of eDNA in this process have yet to be elucidated. We have used advanced techniques in microscopy, computer vision and image informatics to explore the roles of eDNA during early biofilm development and during active expansion of biofilms formed by *P.aeruginosa*. Many species of bacteria, including *P. aeruginosa* utilize type IV pili mediated twitching motility to actively translocate across solid and semi-solid surfaces. Twitching motility can manifest as a complex, multicellular behavior that enables the active expansion of bacterial biofilms. Under appropriate conditions, such as those encountered at the interface of a glass coverslip and semi-solid nutrient media, the expanding biofilm can develop dramatic networks of intersecting trails. Our analyses reveal that at the leading edge of the interstitial biofilm, highly coherent groups of bacteria migrate across the surface of the semi-solid media, and in doing so, create furrows along which following cells preferentially migrate. This leads to the emergence of a network of trails that guide mass transit toward the leading edges of the biofilm. We have determined that eDNA facilitates efficient traffic flow throughout the expanding biofilm by maintaining coherent cell alignments, thereby avoiding traffic jams and ensuring an efficient supply of cells to the migrating front. Our analyses reveal that eDNA also co-ordinates the movements of cells in the leading edge rafts and is required for the assembly of cells into aggregates that forge the interconnecting furrows. Our observations have revealed that large-scale self-organization of cells in actively expanding biofilms of *P. aeruginosa* occurs through construction of an intricate network of furrows that is facilitated by eDNA.

5:20pm **BI+AI+BA+IS-MoA11 Sample Preparation and Optimization for Bacterial Identification by Raman Spectroscopy**, ***M.M. Hlaing***, *M. Dunn, S.M. McArthur, P.R. Stoddart*, Swinburne University of Technology, Australia

The characterisation and identification of individual bacteria using Raman spectroscopy can aid in rapid, in situ microbiological diagnosis and hence timely, appropriate treatment and control measures [1, 2]. Appropriate sample preparation methods and experimental conditions are crucial to avoid some potential difficulties in analysing the information-rich Raman spectra from bacterial cells. In this study, the Raman spectra of fresh and stored samples of bacterial isolates (*Escherichia coli*) were analysed to determine any variations caused by sample processing. Analysis based on principal components suggests that different methods of sample preparation and storage affect the spectral components associated with different biochemical compounds in bacterial cells. The effect of long term storage in glycerol stock at freezing temperatures on the Raman spectrum of cells from the early exponential phase was observed in this study and found to modify the bacteria cells. Furthermore, the presence of extracellular polymeric substance (EPS) matrix around bacterial cells at later stages of the growth cycle provide higher resistance to environmental stress compared with other phases. Based on these results, a specific experimental protocol has been developed in order to obtain interpretable, comparable and reliable Raman data from bacterial samples.

Keywords: Raman spectroscopy; Bacterial identification; Sample preparation.

References

[1] W. E. Huang, R. I. Griffiths. Anal. Chem. 2004, **76**(15): 4452-4458.

[2] T. J. Moritz, S. T. Douglas. J. Clin. Microbiol. 2010, **48**(11): 4287-4290.

Biomaterial Interfaces
Room: 201 B - Session BI+AS+BA+NL-TuM

Biointerface, Energy and Environmental Applications of QCM
Moderator: L. Hanley, University of Illinois at Chicago

8:00am **BI+AS+BA+NL-TuM1 QCM-D for Energy and Environmental Applications**, ***B.H. Kasemo***, Chalmers University of Technology, Sweden **INVITED**

QCM-D has over the past ca. 15 years matured to a measurement technique with a manifold of applications for liquid or gas phase applications. "D" stands for *dissipation* or damping of the sensor oscillation. It yields new information about sample visco-elastic properties, in addition to the mass changes at the ng/cm^2 level obtained from the QCM frequency shift. New information is obtained when the overlayer or film that is studied, causes significant energy dissipation. This is e.g. the case with viscous or visco-elastic films and molecular adlayers. In such cases the two independent quantities, the frequency shift Δf and the dissipation change ΔD , via modeling, allow unique new information to be extracted from the measurements, compared to conventional QCM. In addition, the magnitude of ΔD provides an immediate hint if the Sauerbrey relation, converting Δf to a proportional change in mass, is applicable or not. Major application areas of QCM-D in the past and currently are biomolecule adsorption on surfaces, e.g. on medical implant materials, supported lipid bilayers mimicking cell membranes, polyelectrolytes e.g. layer-by-layer growth, polymer coatings and their curing and phase changes, and more recently cell and bacterial studies. Well over 1200 QCM-D publications have been produced in these areas, cited over 15 000 times. More recently studies related to applications in the energy and environmental areas have rapidly increased. Energy technology examples include solar cells (dye impregnation of DSSC), fuel cell electrode corrosion, studies related to fossil fuel properties and processes, hydrogen storage and CO2 capture/sorption. In the environmental area many applications relate to nanoparticle safety and toxicity, e.g. measuring (surface) affinities between NPs and other materials or agglomeration between NPs. Yet another growing area is to use supported lipid membranes as up-stream model and screening systems, mimicking cell membranes, for testing of NP affinity to such membranes. The method is also used for other aspects of waste water cleaning, such as measuring affinities to filtering materials and membranes of heavy metal ions and other impurities.

9:00am **BI+AS+BA+NL-TuM4 Accounting for Unintended Binding Events in the Analysis of Quartz Crystal Microbalance Kinetic Data**, ***G. Heller***, *T. Zwang, M. Sazinsky, A. Radunskaya, M.S. Johal*, Pomona College

Previous methods for analyzing protein-ligand binding events using the Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) fail to account for unintended binding that inevitably occurs during surface measurements and obscure kinetic information. In this talk, I present a system of differential equations that accounts for both reversible and irreversible unintended interactions. This model is tested on three well-characterized protein-ligand systems, each of which has different features, to establish the feasibility of using the QCM-D for protein binding analysis. The first system presented is the binding of hemin to human serum albumin. The second is the binding of Fe (III) 2,5-dihydroxybenzoic acid complex to neutrophil gelatinase-associated lipocalin tagged with glutathione S-transferase. The third system presented is the interaction of caffeine and bovine serum albumin. Characteristics of the QCM-D binding data for these three systems that are inconsistent with previous QCM-D kinetic models are 1) a non-constant deposition rate in the association phase, 2) a non-zero mass near the steady state of the rinse phase, 3) a non-linear dependence on ligand concentration, and 4) a non-constant ligand concentration for runs lasting short periods of time. Our model accounts for these factors and demonstrates the feasibility of using QCM-D to extract kinetic information and accurately determine affinity constants (K_d) for protein-ligand complexes.

9:20am **BI+AS+BA+NL-TuM5 Silica Nanoparticle – Lipid Membrane Interaction Studies Towards Nano(Q)SAR?**, *L. De Battice, R. Frost,* Chalmers University of Technology, Sweden, *A. Sundblom, M. Persson,* AkzoNobel PPC, Sweden, *M. Wallin, J. Sturve,* University of Gothenburg, Sweden, ***S. Svedhem***, Chalmers University of Technology, Sweden

To improve on the performance of silica-based nanomaterials, and to reduce environmental and health risks related to this development, it is important to learn about how engineered nanomaterials interact with e.g. biomolecules and biological barriers. We are also interested in the development of a generic screening methodology for nanoparticles, and to identify nanoparticle features which are likely to lead to effects in cells. The present results have been obtained with a set of five silica nanoparticles, four of which were spherical (about 20 nm in diameter) and one of which had an elongated shape (roughly 4 x 20 nm). Size and zeta potential measurements were performed, and the adsorption profiles for the nanoparticles when interacting with each of four model lipid membranes of different composition and net charge were monitored in real time using the quartz crystal microbalance with dissipation monitoring (QCM-D). We found clear differences in adsorption profiles on the model membranes with respect to surface coating, and particle shape. These results were compared to the results obtained when exposing frog cells to the same particles, using a conventional assay detecting cellular damage and cytotoxicity (through cell lactate dehydrogenase (LDH) release) and as well in experiments where the function of frog cells cultured on QCM-D sensors was studied by QCM-D (the method is published in Frost et al., Analytical Biochemistry, in press). In general, there were small effects on the cells.

The results will be discussed in the perspective of establishing (Q)SAR for nanoparticles.

9:40am **BI+AS+BA+NL-TuM6 Using Real-Time Acoustical Sensing by QCM-D to follow Dynamic Processes in Live Cell Morphology and Cell-Surface Interactions**, ***E. Nilebäck***, Biolin Scientific, Sweden, *N. Tymchenko, A. Kunze,* Chalmers University of Technology, Sweden, *L. Enochson,* University of Gothenburg, Sweden, *P. Wallin, J. Gold, S. Svedhem,* Chalmers University of Technology, Sweden, *A. Lindahl*, University of Gothenburg, Sweden

The mechanical properties and morphology of living cells are dynamic and regulated by cell signaling pathways that can be triggered by both external and internal stimuli. The dynamic nature of these cellular shape changes leaves a great potential for real-time techniques to reveal new time-resolved information in addition to microscopy methods based on fluorescence that are typically end-point measurements. By using quartz crystal microbalance with dissipation monitoring (QCM-D), the nano-mechanical properties at the cell-surface interface can be studied. How the cells interact with the surface greatly influences the QCM-D response, particularly at cell adhesion and when the cells undergo morphological changes due to internal or external stimuli.

To explore the potential of acoustically sensing the cell-surface interface in real-time, we have used QCM-D as the main technique in several cell studies:

i) Changes in cell morphology were studied simultaneously by QCM-D and light microscopy as 3t3 and human derived fibroblasts were subjected to the actin disrupting agent cytochalasin D that depolymerizes actin in the cytoskeleton. This resulted in a dramatic change in cell morphology that was reversible upon rinsing and could repeatedly be detected as significant changes in the energy dissipation. [1]

ii) Cell adhesion and cell-surface interactions were studied for human derived chondrocytes as they were subjected to well-defined layers of the glycosaminoglycan (GAG) hyaluronan (HA). HA is present in e.g. extra cellular matrix of cartilage and the chondrocytes could be seen in the QCM-D signal to degrade the GAG layer in 2 hours.

iii) Cell adhesion and fixation studies of 3t3 fibroblasts were performed on silicon dioxide coated surfaces with and without a coating of serum proteins. This revealed that the protein layer greatly affected the QCM-D response from the cells. The later fixation by formaldehyde was performed *in situ* and from the QCM-D data it was shown that the viscoelastic behavior of the cells was to a large extent retained after fixation.

1. Tymchenko, N., Nilebäck, E. et al., *Reversible Changes in Cell Morphology due to Cytoskeletal Rearrangements Measured in Real-time by QCM-D,* Biointerphases, 2012. (1): p. 1-9.

11:00am **BI+AS+BA+NL-TuM10 QCM-D as a Novel Technique to Investigate Nuclear Pore Transport**, ***M. Sorci***, Rensselaer Polytechnic Institute, *R. Hayama, B.T. Chait, M.P. Rout,* Rockefeller University, *G. Belfort*, Rensselaer Polytechnic Institute

A quartz crystal microbalance (QCM-D) is a simple and highly sensitive mass and dissipation sensor which has been used to study interfacial adsorption reactions and conformational changes on a variety of supports in real time. In this paper we aim to apply this technique to gain a better understanding of nuclear transport. In particular, we are investigating the transport of proteins through the Nuclear Pore Complex (NPC), which is the sole mediator of exchange between the nucleus and the cytoplasm in all

eukaryotic cells[1]. Recent publications have further improved our understanding of the architecture and evolutionary origins of this macromolecular gate,[2,3] yet the molecular transport mechanism remains unclear. Transport across the NPC is fast, energy-dependent (to give directionality) and often receptor-mediated. While small molecules pass through the NPCs unchallenged, large macromolecules (>40 kDa) are excluded unless chaperoned across by transport factors collectively termed Karyopherins (Kaps). The translocation of the complexes of Kaps and their cargo proteins/RNAs occurs through the specific affinity and binding between Kaps and particular nuclear pore complex proteins (nucleoporins) called FG-Nups, which share a degenerate multiple-repeated "Phe-Gly" motif. In an attempt to better understand the transport and the selective process under crowding conditions, we immobilized Nsp1 and truncated variations of it onto QCM-D sensors. The binding and unbinding of Kap95, other binding proteins, as well as control proteins (e.g. BSA), was studied in order to investigate specificity, kinetics rate constants, effect of competitive binding. Ultimately we aim to gain sufficient understanding of the molecular scale engineering principles behind nuclear transport to allow us to design the next generation of synthetic selective nanosorters capable of purifying any protein that we desire.

1. Grünwald, Singer and Rout, Nature 2011, 475, 333

2. Alber et al., Nature 2007, 450, 683

3. Alber et al., Nature 2007, 450, 695

11:20am **BI+AS+BA+NL-TuM11 Using QCM-D and Ellipsometry to Determine the Orientation and State of Hydration of Antibodies Adsorbed on a Hydrophobic Surface**, ***C.W. Frank***, Stanford University, *M.E. Wiseman*, DSM Research **INVITED**

Adsorbed antibodies can take several orientations: end-on/fab-up, end-on/fab-down, side-on, and flat-on. Since the accessibility of antigens will depend on the antibody orientation, we have used QCM-D to monitor transient adsorption and have determined the orientation as a function of coverage. In addition, we have used simultaneous QCM-D and ellipsometry to distinguish between the "wet" mass consisting of protein plus coupled water and the "dry" mass consisting only of the protein. Finally, we have applied an alternative protocol for determining the state of hydration using only QCM-D. This involves a D_2O exchange that allows determination of the dry mass. We conclude that the QCM-D signal of proteins in liquids contains a major component from coupled water.

Manufacturing Science and Technology
Room: 202 B - Session MS+AS+BA+BI+PS+TF-TuM

IPF 2013-Manufacturing Challenges for Emerging Technologies: IV. Manufacturing Challenges: The Life Sciences

Moderator: D.G. Castner, University of Washington, L.J. Gamble, University of Washington

8:00am **MS+AS+BA+BI+PS+TF-TuM1 Microfluidics for Chemical Analysis**, ***L. Carr***, *Q. Bai, R. Brennen, S. Post, G. Staples, K. Seaward, H. Yin, L. Martinez, D. Ritchey, K. Killeen*, Agilent Technologies **INVITED**

Chemical analysis is an essential tool for pharmaceuticals, environmental testing, food safety, forensics, energy and many other industries. The need for faster, more accurate and more sensitive measurements continuously pushes the limits of measurement technology and creates opportunities for advances in chemical analysis instruments and applications. One way in which this need can be addressed is by incorporating microfluidic devices in High Pressure Liquid Chromatography (HPLC). Pressure-based microfluidic chips have enabled a new class of reproducible integrated workflow devices that combine sample preparation, enrichment, and HPLC separation *with an integrated ESI/MS (Electrospray Ionization/Mass Spectrometry) interface* for high sensitivity nanoflow Liquid Chromatography-Mass Spectrometry (LC-MS). These devices have most commonly been fabricated using polymer, ceramic, and glass materials but the next generation of higher capacity and throughput microfluidic chips for LC-MS requires materials and structures capable of ultra high pressure operation. In this work, we describe the fabrication and performance of diffusion-bonded metal chips for high performance nano- and microflow LC-MS operation. The microfabrication technology required to make these devices includes semiconductor fabrication standards such as photolithography and thin film deposition, as well as laser ablation, electrochemical etching, and diffusion bonding. These novel metal devices exhibit state of the art performance in resolution and throughput for microfluidic LC-MS chips. These chips are an example of improvements in measurement sensitivity, resolution, speed, and ease of use that have been made possible by utilizing microfluidic devices for chemical analysis.

8:40am **MS+AS+BA+BI+PS+TF-TuM3 Challenges in the Fabrication of Nanoscale Devices for DNA Base Sensing**, ***S. Papa Rao***, *J. Bai, E.A. Joseph, R.L. Bruce, M. Lofaro, M. Krishnan, M. Brink, M. Guillorn, S.M. Rossnagel, Q. Lin, J. Cotte, C. Jahnes, Smith, Gignac, Reuter, Nam, Astier, Wang, Stolovitsky, Goldblatt*, IBM Research Division, T.J. Watson Research Center **INVITED**

The fabrication of integrated circuits with increasingly fine geometries has required the development of advanced process technologies, which can be further refined for the purpose of building devices for biological applications. Applications such as sensing nucleotides in DNA require structures that are of the order of a few nanometers. This talk will focus on the specific challenges encountered in the fabrication of such nano-scale devices – broadly classified into materials-related challenges, unit-process challenges and process integration-related challenges. Issues such as dielectric integrity, metal recrystallization, and materials compatibility with chemistries used down-stream will be discussed. Dimension control during fabrication of ~10 nm sized structures was achieved through intense process development efforts of reactive ion etch and chemical mechanical planarization (both manufacturing-friendly techniques). Device layout issues that affect manufacturability will be presented. Finally, some of the important lessons learned in achieving a high yield of reliable devices through process-integration changes will also be discussed.

9:20am **MS+AS+BA+BI+PS+TF-TuM5 Nucleic Acid Synthesis and Applications**, ***S. Laderman***, Agilent Technologies **INVITED**

The pursuit of perfect and practical *de novo* chemical syntheses of nucleic acids has been the foundation of a broad range of life science accomplishments over many decades in the past. Its further pursuit is enabling a broad range of opportunities many decades into the future. These themes will be elucidated by examining the precedents and improvements enabling high throughput genomics for research and diagnostics through the manufacturing of high quality DNA microarrays and complex pools of long oligonucleotides. Looking forward, new ways to synthesize RNA will enable deeper understanding and improved manipulations of cells, tissues and organisms. At the same time, multiple applications of synthetic biology are motivating additional focus on further advances in flexibly and cost-effectively constructing perfect DNA.

10:40am **MS+AS+BA+BI+PS+TF-TuM9 Single Molecule, Real-Time DNA Sequencing**, ***S. Turner***, Pacific Biosciences **INVITED**

In this talk, I'll convey the story of the development and commercialization of Pacific Biosciences' Single Molecule, Real-Time DNA Sequencing technology. I will start with an overview of the method, how it works, and how if differs from sequencing methods that came before it. I will continue with a discussion of some key technology milestones, with an emphasis on the technological advances in materials engineering and nanofabrication. I'll finish by showing some examples of how this technology has transformed the field of DNA sequencing and genome analysis.

11:20am **MS+AS+BA+BI+PS+TF-TuM11 Opportunities and Challenges in the Biobased Products Manufacturing**, ***J. Flatt***, *S. Bailey, S. Bower, D. Gibson, S. Farah, J. Butler, J. Hannon*, Synthetic Genomics **INVITED**

Biobased production of life's necessities, including food, fuels, chemicals and medicines provides a foundation for sustainable and geographically distributed manufacturing processes. Biobased manufacturing utilizes photosynthetic processes directly through conversion of carbon dioxide and light energy or indirectly through conversion of renewable biomass feedstocks to products. Biological cells (biocatalysts) are the operating systems for these biobased manufacturing processes. Rapid advances in synthetic biology enable the engineering of biocatalysts which can produce a broader range of products than previously possible, at high yields and productivities necessary for achievement of desired economics. Improvements in biocatalysts are achieved through modifications of DNA, which is the software of living systems. Significant advances in the costs, fidelity and speed of DNA synthesis, along with improving understanding of gene function and regulation is enabling the more rapid development of biocatalysts which achieve required performance for commercially viable manufacturing processes. The current state of the art of synthetic biology and technology trends which will impact future development of biobased processes will be discussed. Additional market-specific and process-specific challenges exist, and will be discussed in context of the specific examples taken from manufacture of synthetic vaccines, biobased chemicals and fuels. Recently, Novartis and Synthetic Genomics demonstrated the ability to successfully produce vaccines for prevention of seasonal influenza using synthetic DNA constructs, which significantly reduces the time from

influenza strain identification to production of the vaccine seed. Development of this revolutionary process required significant improvement of the fidelity of DNA synthesis and assembly, which provides insight into the challenge of engineering more complex biocatalysts. On the other end of the spectrum, phototrophic microalgae have great long-term potential to provide a sustainable and alternative source of food and liquid transportation fuels. Phototrophic microalgae can be cultivated using non-potable water on non-arable land. Techno-economic analysis (TEA) and life cycle assessment (LCA) both suggest that significant improvements in biocatalyst productivity and capital cost reduction will be required to achieve competitive economics. Maximum observed algal biomass productivities in the range of 20 to 25 g/m2/day are far lower than generally-agreed upon theoretically-achievable productivities based upon the actual solar energy available. Improvement of photosynthetic efficiency in mass culture is required for economical algal-based processes. Limited availability of light in mass culture also limits the maximum achievable cell density, which results in increased downstream processing costs. The challenges of "dilute solution economics" associated with commercial algae production and potential biological and engineering solutions will be discussed.

Ions at Aqueous Interfaces Focus Topic
Room: 201 B - Session IA+BA-TuA

Ions and Biomolecules at Aqueous Interfaces

Moderator: J.M. Gibbs-Davis, University of Alberta, Canada

2:00pm **IA+BA-TuA1 Selective Adsorption of Ions to Aqueous Interfaces and its Effects on Evaporation Rates**, ***R.J. Saykally***, University of California, Berkeley **INVITED**

By exploiting the strong charge-transfer-to-solvent (CTTS) resonances of selected anions in aqueous electrolytes, their interfacial adsorption properties are measured by UV-SHG spectroscopy. Temperature and concentration dependences are determined, with the goal of establishing a complete molecular description of selective ion adsorption. A study of thiocyanate reveals that its strong adsorption is driven by hydration forces and impeded by a novel entropy effect. A study of nitrite indicates adsorption as an ion pair with sodium. Evaporation rates are measured by combining liquid microjet technology and Raman thermometry. The relationship between surface propensities of ions and evaporation rates is investigated. A detailed molecular mechanism for both selective ion adsorption and aqueous evaporation is explored.

2:40pm **IA+BA-TuA3 Exploring Ion Interactions at Aqueous Interfaces**, ***P.S. Cremer***, Penn State University **INVITED**

We have employed a combination of surface specific techniques to interrogate the interactions of ions with self-assembled monolayers and proteins at aqueous interfaces. The results provide direct insight into ion pairing interactions. In particular, I will discuss the behavior of cations and anions as they relate to the Hofmeister series, which is a rank ordering of the efficacy of these species to influence the physical behavior of colloidal and interfacial systems in solution. The TiO_2/water, quartz/water, alkyl chain/water, and air/water interfaces were each explored.

Experiments consisted of a combination of sum frequency generation and thermodynamic measurements. Ion specific effects at these interfaces were found to be determined by several factors. These include the sign and magnitude of the surface potential, ion pairing effects, as well as the presence of polar and nonpolar interfacial moieties. At negatively charged, hydrophilic surfaces, we found that Na^+ adsorption and double layer formation was modulated by the nature of the counterion in solution. For the anions, it was found that SCN^- was less depleted at the interface compared with better hydrated anions such as Cl^-. The same ordering was observed for the anions whether this interface was relatively hydrophobic or hydrophilic. Changing the sign of the charge at the interface also led to a similar Hofmeister ordering. Curiously, the ordering for cations at these aqueous interfaces was found to be more sensitive to the specific surface chemistry. Moreover, at negatively charged hydrophilic surfaces, the smallest and best hydrated cations were mostly favored over more poorly hydrated cations. By contrast, well hydrated cations were repelled from more apolar surfaces. Li^+ displayed somewhat anomalous behavior. All of these results will be discussed with an eye toward a broader model for interfacial partitioning of ions in aqueous solutions.

4:00pm **IA+BA-TuA7 Revealing the Dynamics of Lipid Composition in Phospholipid Bilayers by Sum-Frequency Vibrational Spectroscopy**, ***J. Conboy***, University of Utah **INVITED**

A membrane, only two molecules thick, surrounds all cells and is responsible for controlling the passage of materials in and out of the cell in a selective manner. Our current understanding of the structure and dynamics of cellular membranes emerged in the early 1970's. However, there is still much we do not know about this seemingly simple "shell" which makes life as we know it possible. For example, the location of the negatively charged phosphatidylserine (PS) headgroup lipids has drastic effects on cell function, ranging from coagulation to apoptosis. The localization of PS in one leaflet of the membrane is governed by a complex interplay between kinetic and thermodynamic factors. However, the kinetics of PS exchange has not been studied in detail. Using methods of classical surface chemistry coupled with nonlinear optical methods, we have developed a novel analytical approach, using sum-frequency vibrational spectroscopy (SFVS), to selectively probe lipid compositional asymmetry in a planar supported lipid bilayer. SFVS has been used to measure both the compositional asymmetry and kinetics of PS and phosphatidylcholine (PC) lipid flip-flop in planar supported lipid bilayers composed of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-dihexadecanoyl-sn-glycero-3-phospho-L-serine (DPPS). The transition state thermodynamics of DSPC and DPPS were measured at biologically relevant compositions ranging from 10 to 35 % DPPS. The activation thermodynamics of DSPC and DPPS and their impact on compositional asymmetry will be discussed in detail.

4:40pm **IA+BA-TuA9 Characterization of Protein Secondary Structures at Interfaces Using Chiral Sum Frequency Generation**, ***C.Y. Yan***, Yale University **INVITED**

Characterization of protein secondary structures using vibrational spectroscopy is challenging because of strong vibrational background from water and spectral overlapping of vibrational signatures for various secondary structures. Here, we present chiral vibrational spectra of amide I and N-H stretch of protein backbone in various secondary structures at interfaces obtained by chiral sum frequency generation (SFG) spectroscopy. These spectra show unique signatures for parallel beta-sheets, anti-parallel beta-sheets, alpha-helices, 3-10 helices, and random-coils. Because the chiral SFG spectra are muted to achiral solvent, the N-H stretch can be detected at zero water background. Thus, the N-H stretch frequency can probe local H-bond environments, providing an additional signature to distinguish secondary structures. This allows chiral SFG to resolve secondary structures at interfaces, such as alpha-helices versus 3-10 helices, which elude conventional vibrational methods and circular dichroism spectroscopy. Hence, chiral SFG holds promises to address fundamental and engineering problems in biomedical and material sciences.

5:20pm **IA+BA-TuA11 Order Matters – Detecting Non-Isotropic Structures in Complex Biological Samples**, ***P. Koelsch***, University of Washington

Order is omnipresent in biological systems in various forms and on almost all length-scales. Here we discuss how to use order as a (label-free) contrast mechanism in microscopy or selectivity criteria in surface spectroscopy to detect and analyze non-isotropic arrangements in complex *in vitro* scenarios. Examples are fibrillar structures that can be visualized within tissue via second-harmonic-generation (SHG) microscopy or detected on surfaces via vibrational sum-frequency-generation (SFG) spectroscopy. The contrast mechanism in SHG microscopy is order and similarly is order (and chirality) the selectivity criteria when is comes to SFG spectroscopic measurements on surfaces. Examples to be discussed are fibrillar arrangements within the extracellular matrix of adherent cells on substrates or within cancerous tissue samples.

5:40pm **IA+BA-TuA12 Aqueous Solution Chemistry Studied by Soft X-ray Absorption Spectroscopy**, ***T.Z. Regier***, Canadian Light Source, Canada, *C. Phillips, D. Peak, R. Green, A. Moewes, J. Tse,* University of Saskatchewan, Canada, *A. Achkar, D. Hawthorn*, University of Waterloo, Canada

X-ray absorption spectroscopy is a sensitive probe of transition metal co-ordination and bonding environment. Excitation of 2p electrons into unoccupied 3d orbitals allows for determination of crystal field parameters and ligand field strength. Measurement of the x-ray absorption spectra of Cu and Fe ions in solutions was performed using a continuous flow cell on the SGM beamline at the Canadian Light Source. Fluorescence yields and inverse partial fluorescence yields were measured using a multielement silicon drift detector. The interaction between Cu ions and various organic ligands was studied and the difference between the absorption and fluorescence intensities was examined for aqueous ferrous and ferric solutions.

Biomaterial Interfaces
Room: 201 B - Session BI+AI+AS+BA+IA+NL+NS+SP-WeA

Characterization of Biointerfaces
Moderator: A. Rosenhahn, Ruhr-University Bochum, Germany

2:00pm **BI+AI+AS+BA+IA+NL+NS+SP-WeA1 Barrier Properties of the Three Layers of the Stratum Corneum to Metal Ions Analyzed by TOF-SIMS**, *I. Ishizaki*, ULVAC-PHI, Inc., Japan, ***J.S. Hammond***, Physical Electronics Inc., *A. Kubo, H. Kawasaki, K. Nagao*, Keio University, Japan, *Y. Ohashi*, ULVAC-PHI, Inc., Japan, *M. Amagai, A. Kubo*, Keio University, Japan

The stratum corneum (SC) is the outermost barrier protecting the mammalian body from desiccation and foreign insults. Congenital SC barrier insufficiencies, i.e., filaggrin deficiency, are hypothesized to predispose patients to atopic diseases. The insoluble nature of the SC has hampered in-depth-analysis of its barrier function by conventional cell biological methods. Here, we applied time-of-flight secondary-ion-mass-spectrometry (TOF-SIMS) imaging technology to analyze the SC in skin sections of wild type and filaggrin knockout mice.

TOF-SIMS enabled the visualization of the distribution of natural substances and the infiltration of externally applied molecules directly without any staining procedure. The distribution of potassium (K) and arginine revealed that the SC consists of three sharply demarcated layers. K was concentrated in the upper layer, while arginine, a major component of the filaggrin-derived natural moisturizing factors, was specifically concentrated in the middle layer and markedly decreased in the filaggrin knockout SC. When skin was soaked in water, K of the upper layer disappeared. When the mice tails were soaked in solutions of K or hexavalent chromium before cross-sectioning, the TOF-SIMS line scan data indicates that the upper layer of the SC allowed the influx of these ions, suggesting that this layer acts like a "sponge" allowing the passive influx and efflux of exogenous ions. The middle layer blocked the influx of K and hexavalent chromium ions, but failed to block the influx of trivalent chromium ions, which was blocked at the lower layer. Therefore the middle and lower layers have distinct barrier properties depending on each metal. Filaggrin deficiency resulted in the abrogation of the lower layer barrier, allowing trivalent chromium to permeate through the SC to viable epidermal layers. These results, obtained by TOF-SIMS analyses, reveal that the SC consists of three layers of distinct functional properties and demonstrate the loss of barrier properties for particular metal ions in filaggrin deficient SC samples.

2:20pm **BI+AI+AS+BA+IA+NL+NS+SP-WeA2 Imaging Hydrated *Schewanella p.* Biofilm in a Microfluidic Reactor by ToF-SIMS**, ***X.Y. Yu***, *M. Marshall, B. Liu, Z. Zhu, L. Yang, E. Hill, S. Belchik*, Pacific Northwest National Laboratory

We recently developed a microfluidic interface that enables direct probing of liquid surface in vacuum using ToF-SIMS and SEM. The device contains a 100 nm thick silicon nitride (SiN) membrane as the detection area (1.5 ´ 1.5 mm^2) and the microchannels fabricated from polydimethylsiloxane (PDMS) using soft lithography. The unique aspect of our approach is that the detection window is an aperture of 2-3 m m diameter, which allows direct detection of the liquid surface and use surface tension to hold the liquid within the aperture. Its application in ToF-SIMS as an analytical tool was evaluated. In this paper, we present new results of using the microfluidic flow cell to grow *Schewanella p.* biofilm and characterize the biofilm subsequently using ToF-SIMS in the hydrated environment. Depth profiling was used to drill through the SiN membrane and the biofilm grown on the substrate. A controlled media sample was used to compare with the wet biofilm sample. In addition, dry samples deposited on clean silicon wafer were studied to show the difference between wet and dry samples. Multivariate statistical analysis including Principle Component Analysis was used to investigate observations. Our results indicate that imaging biofilm in the hydrated environment using ToF-SIMS is possible using the unique microfluidic device for the first time. Moreover, characteristic biofilm fragments were observed in the wet sample than in dry sample, illustrating the advantage of imaging biofilm in the hydrated state.

2:40pm **BI+AI+AS+BA+IA+NL+NS+SP-WeA3 High-resolution Secondary Ion Mass Spectrometry Imaging of Distinct Lipid Species in the Plasma Membranes of Mammalian Cells**, ***M.L. Kraft***, University of Illinois at Urbana Champaign **INVITED**

The plasma membrane is the selectively permeable lipid bilayer that separates every cell from its surroundings. In mammalian cells, the plasma membrane contains domains of differing protein composition. Growing evidence suggests that each different lipid species and cholesterol are also organized into compositionally and functionally domains within the plasma membrane. Domains that are enriched with cholesterol and sphingolipids, which are often referred to as lipid rafts, are hypothesized to be present in the plasma membrane and influence its functions. Despite this potential importance, the organizations of cholesterol and sphingolipids in cell membranes are poorly understood. Until recently, the distributions of most lipid species could not be directly imaged without the use of fluorophore labels, which may alter the distributions of the lipid molecules that they label. We have combined high-resolution SIMS, which is performed with a Cameca NanoSIMS 50, with metabolic stable isotope labeling in order to visualize the organizations of rare isotope-labeled lipids in the plasma membrane by mapping their distinctive isotope enrichments. Here, the details of this approach and its application to imaging the distributions of metabolically incorporated ^{15}N-sphingolipids and ^{18}O-cholesterol in the plasma membranes of fibroblast cells will be presented. Use of this approach to evaluate hypotheses concerning the mechanisms that regulate lipid organization within the plasma membrane will also be discussed.

4:00pm **BI+AI+AS+BA+IA+NL+NS+SP-WeA7 Analysis of Breast Cancer Tumors with ToF-SIMS**, ***L.J. Gamble***, *M. Robinson*, University of Washington, *F. Morrish, D. Hockenbery*, Fred Hutchinson Cancer Research Center

Tumor metabolism plays a large role in cancer onset and progression, and its causes and effects are under intense scrutiny. Recently, the lipid metabolism in tumors has been looked at as a factor in tumor type and treatment. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is well suited for analysis of the lipid distribution in tumors. In this study, frozen breast cancer tissue specimens from patients were cut using a cryomicrotome at a thickness of 4µm and deposited on silicon wafers. Serial tissue slices were stained with hematoxylin and eosin (H&E) and were used to determine from which structures the various chemical signatures originated. SIMS tissue sample data were acquired on an IONTOF TOF.SIMS V using Bi_3^+ in both high mass and high spatial resolution modes on both ER+ and ER- human breast tumor tissue samples. Mass fragments spectra from multiple spots and tissue slices for the ER+ and ER- tissue samples can be separated from one another using PCA within a 95% confidence interval. Key differences between tissue types are abundance of cholesterol and triacylglycerides/diacylglycerides (TAGs/DAGs). Imaging ToF-SIMS of theses samples show variances for different fatty acids (saturated versus unsaturated) that correlate with model studies using similar cancer cell types.

4:20pm **BI+AI+AS+BA+IA+NL+NS+SP-WeA8 Tools For TOF-SIMS Image Analysis**, ***D.J. Graham***, *L.J. Gamble, D.G. Castner*, University of Washington

The use of time-of-flight secondary ion mass spectrometry (ToF-SIMS) for imaging has increased in recent years. This is due to the improvements in spatial resolution and ion yields from modern primary ion sources. These improvements have made ToF-SIMS attractive for cell and tissue imaging, especially due to the fact that ToF-SIMS can detect and identify a wide range of membrane lipids and other cellular components, and can potentially image these in both 2D and 3D. Characterization of tissues and cells by ToF-SIMS often requires advanced data collection and analysis methodologies including the use of stage rastering for large area analysis and 3D depth profiling. It is also often of interested to localize specific areas within a cell or tissue and carry out region of interest (ROI) analysis. Finally, ToF-SIMS image analysis presents challenges due to the shear size of the data sets. In order to deal with these large, complex data sets, we have created a set of Matlab toolboxes for multivariate analysis of both images and spectra. This talk will highlight new tools in the NBtoolbox that enable the user to process stage raster images, overlay images, and extract ROI images based off of image masks created from any imported image.

For example, the stage raster tools enable the user to import and run PCA on an entire stage raster image, or to dice the stage raster into separate image tiles that can then be analyzed individually. The ROI generation tools enable the user to import any image to be used as a ROI mask. Examples will be shown using florescent images from confocal microscopy as masks to extract ROI from ToF-SIMS images of mouse muscle tissue. Tools are

also included for image alignment, and image cropping. All data processed with these tools can be analyzed using PCA, MAF or MCR.

4:40pm **BI+AI+AS+BA+IA+NL+NS+SP-WeA9 How Hydration Affects Mechanical Anisotropy, Nano-Topography and Fibril Organization of Osteonal Lamellae**, *A. Faingold*, ***S.R. Cohen***, Weizmann Institute of Science, Israel, *R. Shahar*, Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, *S. Weiner, H.D. Wagner*, Weizmann Institute of Science, Israel

Water serves a central role in physiological systems. Even bone, a relatively "dry" component, has high water content: cortical (also known as compact) bone contains about 20% water by volume. The water content varies with age, and influences the structural and mechanical properties of the bone, from the level of mineralized fibrils up to osteonal lamellae. Many studies on mechanical properties of bone are performed on bone which has been dehydrated to some degree, whereas the relevant physiological state is wet. In this work, atomic force microscopy, nanoindentation, and microindentation have been applied to wet and dry bone samples in order to investigate the influence of hydration at different hierarchical levels; the mineralized fibril level (~100nm), the lamellar level (~6µm); and the osteon level (up to ~30µm). Measurements were made both in directions parallel and perpendicular to the osteonal axis by cutting appropriate slices from a metacarpal bone of a 5 year old male horse. "Dry" samples were obtained by allowing the polished sample to stand under ambient conditions for 24 hours. "Wet" samples were measured under deionized water, or PBS solution in which they were incubated between 1 - 18 hours prior to measurement. We note that under these conditions, the wet samples contained 12% water whereas dry samples contained 9% water. Nonetheless, significant differences between the two states were observed: (1) Dry samples were both stiffer and harder than the wet samples in both directions studied, and at all length scales. (2) The anisotropy ratio, ratio of modulus or hardness along vs. perpendicular to the osteonal axis, was larger in the dry samples than for the wet ones. (3) These mechanical changes are accompanied by marked variation in the sample topography as observed by atomic force microscopy. These results will be presented in the context of related work. A model we developed based on differences in the fibril orientation between dry and wet states provides a good rationale for the observed behavior.

5:00pm **BI+AI+AS+BA+IA+NL+NS+SP-WeA10 AFM of Supported Lipid Bilayers: From Critical Point Behaviour to Actin Polymerization**, ***G.R. Heath***, *S.D. Connell, S.D. Evans*, University of Leeds, UK

In this study we create supported lipid model membranes which display phase separation into liquid-ordered and liquid disordered domains and use atomic force microscopy (AFM) to observe critical phenomena and protein interactions with the aid of stable and precise temperature control. The regions of criticality were determined by accurately measuring and calculating phase diagrams for the 2 phase L d –L o region, and tracking how it moves with temperature, then increasing the sampling density around the estimated critical regions. Compositional fluctuations were observed above the critical temperature (T c) and characterized using a spatial correlation function. From this analysis, the phase transition was found to be most closely described by the 2D Ising model, showing it is a critical transition. The region of critically fluctuating 10–100 nm nanodomains has been found to extend a considerable distance above T c to temperatures within the biological range, and seem to be an ideal candidate for the actual structure of lipid rafts in cell membranes. Although evidence for this idea has recently emerged, this is the first direct evidence for nanoscale domains in the critical region.

Ponticulin is a 17KDa integral membrane protein with multiple membrane spanning beta strands and glycosylphosphatidylinositol (GPI) lipid anchor at its C-terminus. Ponticulin has been shown to be the major high affinity link between the plasma membrane and the cortial actin network in *D. discoideum* (Wuestehube and luna, 1987; Chia et al., 1991). This protien in thought to reside in cholesterol-rich lipid microdomains (‘lipid rafts’) with the transmembrane domain apparently lying outside the lipid raft with the raft localization being dependant upon the GPI anchor at the C-terminus of the protein. We test the hypothesis of localization and show for the first signs of GPI-anchored membranes proteins preferentially locating to boundaries between the lo and ld phase. This may provide a potential mechanism by which the cytoskeleton can influence lipid organization.

Cationic lipids have been previously shown to adsorb actin from a non polymerizing solution, induce its polymerization, and form a 2D network of actin filaments, in conditions that forbid bulk polymerization. We show this phenomenon on supported lipid bilayers using high resolution AFM and QCM-D, investigating various factors such pH, charge concentration and lipid mobility which affect the actin structures formed. We then go on to mathematically model this process to show 2 different polymerization mechanisms depending on the lipid diffusion.

5:20pm **BI+AI+AS+BA+IA+NL+NS+SP-WeA11 Investigating Reversible Dye Adsorption on TiO_2: A QCM-D Study**, ***H.K. Wayment-Steele***, *L.E. Johnson*, Pomona College, *M.P. Dixon*, Biolin Scientific, *M.S. Johal*, Pomona College

Understanding the kinetics of dye adsorption on semiconductors is crucial for designing dye-sensitized solar cells (DSSCs) with enhanced efficiency. Harms et al. (2012) have recently demonstrated the applications of QCM-D to show in-situ dye adsorption on flat TiO2 surfaces. QCM-D provides adsorption measurements in real time and therefore determination of the kinetics of the process. In this work, we examine reversible, non-covalent binding of N3, a commercial RuBipy dye, using the native oxide layer of a titanium sensor to simulate the TiO_2 substrate of a DSSC. To isolate the weak binding mode, we deactivated the carboxylate groups of N3 by forming methyl esters, thus disabling chelation to TiO_2. Improved understanding of the weak binding mode provides insight into dye aggregation and the relative contributions of chelation versus non-covalent processes.

5:40pm **BI+AI+AS+BA+IA+NL+NS+SP-WeA12 Combined Raman Systems for Biological Imaging and Analysis**, *A.J. King*, Renishaw Inc, *T. Prusnick*, Renishaw Inc., ***M. Canales***, Renishaw Inc

Raman microscopy has become a routine tool for many materials, but the need for this molecular imaging and analysis technique in biological research has become essential. The ability probe the chemical and molecular structure of biological materials is obtained directly without the need for any dyes or markers. These systems can be utilised to generate chemical images of cells, tissue, bone and bio-compatible materials with very high spatial resolution. It has been employed for cancer diagnosis, stem cell differentiation, skin treatments, protein structure analysis, bio-diagnostics, bacterial identification and green energy.

This Raman instrumentation can also be combined with environmental chambers, scanning probe techniques, scanning electron microscopes and in-vivo probes; to provide in-situ and co-localised measurements. This talk will provide an introduction to Raman microscopy with biological materials; the instrumentation required for these techniques; and, will highlight some applications where Raman microscopy is making the biggest impact with biological materials.

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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