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.

Ions at Aqueous Interfaces Focus Topic
Room: 203 A - Session IA+AI+BI+IS+NL+SS-MoA

Ions at Aqueous Interfaces

Moderator: M.H. Grunze, University of Heidelberg, Germany

2:00pm **IA+AI+BI+IS+NL+SS-MoA1 Sum-frequency Vibrational Spectroscopy for Studies of Ions Emerging at Water Interfaces**, ***Y.R. Shen***, University of California, Berkeley **INVITED**

Ions at water interfaces can significantly change the chemical and physical properties, and hence the functionality, of the interfaces. They play a key role in many important processes in many disciplines. In recent years, sum-frequency vibrational spectroscopy(SFVS) has been demonstrated to be a unique, effective tool to study such interfaces. We discuss here SFVS investigations of ions at various water interfaces: soluble ions at air/water interfaces, ions attached to Langmuir monolayers on water, and hydrophilic and hydrophobic water interfaces. Formation of an electric double charge layer by ions near an interface usually occurs. It induces significant polar reorientation of interfacial water molecules and alters their vibrational spectra that can be detected by SFVS. Useful structural information can be deduced from the results, but work is still needed for complete understanding of the results.

2:40pm **IA+AI+BI+IS+NL+SS-MoA3 Experimental Quantification of Surface Propensity of Halide Ions by Femtosecond Surface Vibrational Spectroscopy**, ***M. Bonn***, Max Planck Institute for Polymer Research, Germany, *H.J. Bakker*, FOM Institute AMOLF, Netherlands, *Z. Zhang, E.H.G. Backus*, Max Planck Institute for Polymer Research, Germany, *L. Piatkowski*, FOM Institute AMOLF, Netherlands **INVITED**

We investigate the vibrational dynamics and energy transfer between interfacial water molecules, in the presence of sodium chloride and sodium iodide salts, using 2-dimensional, femtosecond surface-specific vibrational spectroscopy. We find that both the vibrational lifetime and the intramolecular energy transfer for anion associated interfacial water molecules is slower than for non ion-bound interfacial water molecules. The analysis of the time-dependent slope of the 2-dimensional sum frequency response reveals that the intermolecular resonant energy transfer between the interfacial water molecules is significantly slowed down by the presence of ions. Accordingly, the decay of the frequency-frequency correlation function is slower for NaI than for NaCl solution. This finding provides direct evidence of the higher surface propensity for iodide than for chloride ion, and allows for the quantification of interfacial density of halide ions for both systems.

3:40pm **IA+AI+BI+IS+NL+SS-MoA6 Specific Ion Effects on Acid-Base Equilibria at the Planar Silica/Water Interface**, ***J.M. Gibbs-Davis***, University of Alberta, Canada

The interaction of ions with biological and environmental interfaces depends not only on their valency but also their identity. These specific ion interactions can influence other processes like deprotonation at mineral oxide interfaces. To monitor such interactions we utilized surface specific second harmonic generation (SHG) to report on changes in the surface charge density of silica in real time. We observe that the intrinsic equilibrium constant of the silanol groups is sensitive to the identity of the alkali ion. In contrast, varying the identity of the anion does not affect the intrinsic acidity of the sites but rather their mechanism of deprotonation. Specifically, positive cooperativity is observed in the deprotonation of silanol groups with increasing anion size and polarizability. These results and complementary measurements of the water structure using sum frequency generation spectroscopy will be discussed.

4:00pm **IA+AI+BI+IS+NL+SS-MoA7 Molecular Insight Into the Preferential Adsorption of Monovalent Ions to Selected Polar Surfaces: A Vibrational Sum Frequency Study**, ***E.C. Tyrode***, *R. Corkery*, KTH Royal Institute of Technology, Sweden

Vibrational Sum Frequency Spectroscopy (VSFS) has been used to systematically study the preferential adsorption of a series of monovalent ions to charged and uncharged fatty acid monolayers. Ion enrichment is mainly determined indirectly by targeting surface water vibrational modes. In selected cases however, the ion presence is also directly determined by targeting the fatty acid carboxylate headgroups. A major effort is made to understand the effect of co-ions in the molecular properties of these biophysically relevant interfaces.

4:20pm **IA+AI+BI+IS+NL+SS-MoA8 Dielectric Interfacial Effects**, ***R. Netz***, FU Berlin, Germany **INVITED**

The molecular layer of water molecules on surfaces, the so-called hydration layer, is important for a whole number of properties of biological as well as technological surfaces. Insight can be gained from all-atomistic simulations in conjunction with appropriate continuum modeling.

- Dielectric properties of interfacial water layers are important for the design of high-power capacitors, and can be resolved using simulations.

- At the same time, ions accumulate into a highly condensed interfacial layer, leading to the well-known saturation of the electro-osmotic mobility at large surface charge density regardless of the hydrodynamic boundary conditions. The experimentally well-established apparent excess surface conductivity follows for all hydrodynamic boundary conditions without additional assumptions.

- Hydration water at biological membranes absorbs electromagnetic radiation specifically in the 0.1-10 GHz range that is used for radio communication. Possible health issues are discussed.

5:00pm **IA+AI+BI+IS+NL+SS-MoA10 Liquid Jet –XPS Studies of Ions and Nitriles at the Aqueous Interface**, ***K.A. Perrine***, *M.H.C. Van Spyk, A.M. Margarella,* University of California, Irvine, *H. Bluhm,* Lawrence Berkeley National Laboratory, *B. Winter,* Helmholtz-Zentrum Berlin für Materialien und Energie/Elektronenspeicherring BESSY II, Germany, *M. Faubel,* Max Planck Institute for Dynamik und Selbstorganisation, Germany, *J.C. Hemminger*, University of California, Irvine

Acetonitrile in water is known to exhibit non-ideal behavior. At low concentrations, acetonitrile molecules migrate towards the solution interface leaving water mostly in the bulk. At 0.2 mole fraction, the surface saturates with a full monolayer. Above 0.2 mf, the acetonitrile signal at the surface is enhanced relative to that of the bulk with increasing solution concentration. In the bulk, acetonitrile and water form clusters between 0.2 and 0.7 mole fraction and interact with each other through dipole interactions. Propionitrile, another nitrile with a lower solubility, is also shown have a propensity for the surface of aqueous solutions.

Ions have been shown to impact the properties and solvation structure of aqueous solutions, both at the surface and in the bulk of solution. Potassium iodide (KI) was added to acetonitrile and propionitrile aqueous solutions to observe the effects of ions on nitrile distributions. Liquid jet-X-ray photoelectron spectroscopy (LJ-XPS) was used to characterize the elemental compositions of ions and nitrile species. By tuning the incident photon energy, different depths of the solutions is observed; at low kinetic energies the solution surface is probed and the high kinetic energies the bulk of solution is probed. After adding KI, the interfacial photoelectron spectroscopy signal reveals a reduction in nitrogen and carbon signals in acetonitrile, demonstrating the salting-in effect. With addition of ions to aqueous propionitrile solutions, nitrogen and carbon signals are increased, suggesting a salting-out effect. Sodium chloride ions are also added to aqueous propionitrile studies to determine differences between ions effects from the KI and NaCl salts on propionitrile solutions. These studies help elucidate the role ions play at the interface of aqueous organic solutions.

5:20pm **IA+AI+BI+IS+NL+SS-MoA11 Study of the Structural and Adhesion Forces in Highly Concentrated Electrolytes using Atomic Force Microscopy (AFM)**, ***T. Baimpos***, *M. Valtiner*, Max Planck Institut fur Eisenforschung GmbH, Germany

The understanding of the surface interaction in electrolyte solutions is of paramount importance in many fields such us biology, electrochemistry and surface chemistry. Aqueous solutions of high concentrations are mainly interesting from practical point of view (batteries). In principle, AFM through the Force versus Distance curves (F-D) can be successfully used to probe the electrolyte layering at solid-liquid interfaces and investigate the nature of hydration forces in the presence of various electrolytes of different ion valency, ion concentration or pH [1].

In the current work AFM has been used to measure hydration forces between a non-coated Silicon colloid probe and atomically smooth, flat freshly cleaved Mica surfaces, in highly concentrated monovalent electrolytes (LiCl, NaCl, CsCl). The effect of i) the cation hydration diameter (Li^+>Na^+>Cs^+) and ii) the electrolyte's concentration (0.05-3.0 M), on both the structural (F_{STR}) and adhesion (F_{ADH}) forces are studied. In all environments, F_{ADH} values pass through a minimum as a function of electrolyte's concentration, while for each salt solution, the frequency of structural events is calculated as a function of its concentration. The number of the F-D curves, were classified in appropriate tables according to the number of the structural hydration layers observed. Furthermore, depending on the concentration, 1, 2 or even up to 5 consecutive hydration layers can be clearly distinguished in the same F-D curve from which both the force and the range of each layer can be measured. These results are compared with the hydrated radii of the above ions enabling the extrusion of useful statements concerning the re-arrangement of the structured cation/water layer at the liquid/solid interface.

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.

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