Monday Afternoon, October 28, 2013

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.

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.

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