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+AS+IS+NL-MoM

Surfaces to Control Cell Response
Moderator: H.E. Canavan, University of New Mexico

8:20am **BI+AS+IS+NL-MoM1 Modulation of Cell Behaviour using Self–assembled Binary Colloidal Crystals**, ***P.Y. Wang***, *P. Kingshott*, Swinburne University of Technology, Australia

The control of cell behaviour on surfaces is the key to a broad range of biomedical applications. Biomaterial surfaces with tuneable surface topographies and chemistries can profoundly influence the development of advanced biomaterials used in applications including tissue engineering and regenerative medicine. Recently, we developed an elaborate and feasible method to display an ordered surface topography with tuneable surface chemistry using binary colloidal crystal particles. Using this binary colloidal system, various combinations of particle size and surface chemistry can be readily employed. In this study, two combinations of binary colloidal crystals, i.e. PS-COOH (2 μm)/PMMA (0.4 μm) and SiO_2 (2 μm)/PMMA (0.4 μm) were assembled on ozone-treated silicon wafers. The preliminary results of cell attachment and morphology of L929 fibroblasts and MG63 osteoblasts were studied after 24h.

In general, cells had a small projection area rather than fully spread morphology on the crystal surfaces compared with the flat control. Fibroblasts have abundance of cell protrusions called filopodia which can be observed using scanning electron microscopy (SEM), whilst osteoblasts don't have. Fibroblasts had long and thin extended filopodia on the PS/PMMA crystal surfaces, whilst they had short and thick filopodia on the SiO_2/PMMA crystal surfaces. Regarding the surface chemistry, both SiO_2 and PMMA particles were not as favourable as the PS-COOH particles for fibroblasts attachment, and resulted in the cell projection area on the PS/PMMA being larger compared to the SiO_2/PMMA crystal surfaces. On the contrary, the cell projection area of osteoblasts didn't have significant differences between these two crystal surfaces. After fibronectin coating, cell projection area of osteoblasts on SiO_2/PMMA crystal surfaces increased significantly, whilst fibroblasts didn't, suggesting that different cell types respond to surfaces differently.

These results show for the first time that cell-substrate interactions can be easily controlled by precise positioning of different particles with various sizes and chemistries. The present results will help gain a more thorough understanding of cell-material interactions benefiting the development of advanced biomaterials and materials for tissue engineering.

8:40am **BI+AS+IS+NL-MoM2 Achieving Differential Cell Adhesion with Novel Polymer Surfaces Identified using Microarrays**, ***F.A. Simoes***, *C. Alexander, G. Mantovani, L. Buttery, M.R. Alexander*, University of Nottingham, UK

Stem cells have the ability to repair, replace or regenerate tissues. As a result their potential for regenerative medicine is vast. The processing of cells for therapeutic use and clinical diagnostics will rely on cell sorting steps to ensure a homogeneous population is obtained.[1]

Several techniques exist to achieve this, which rely on the physical properties of cells but tend to provide poor specificity.[2-4] Fluorescence Activated Cell Sorting (FACS) and Magnetic-Activated Cell Sorting (MACS) rely on specific biomarkers. However cells require labelling and label removal steps, which can affect the phenotype.[5]

There is a need for a fully synthetic, inexpensive, label-free separation system, capable of sorting cells with minimum manipulation. In order to generate robust surfaces for such a system, we have developed a method to immobilize thiol-functionalised materials to a polymer substrate using thiol-ene "click" chemistry in a high throughput format. Microarrays of these functionalised polymers comprising of 6 replicates, are fabricated using pin printing to generate a combinatorial library of materials. A mixture of differentiated cells derived from mouse embryoid bodies are then seeded onto the arrays.

Immunohistochemistry techniques are employed to track the differentiation of cells into different lineages, thus enabling the visualisation of multiple cell lines. These techniques also allow for the high throughput quantification of attachment by the means of automatic fluorescence microscopy.

Surface characterisation of the "click" immobilization procedure is performed by X-Ray Photoelectron Spectroscopy. In contrast the characterisation of microarrayed materials is performed using Time of Flight - Secondary ion Mass Spectrometry, which is followed by the ranking of materials using Partial Least Square (PLS) regression analysis. This process allows for the correlation of cell attachment with key molecular ions generated from each material by mass spectrometry.

Successful materials that selectively induce cell attachment are identified and investigated further. This is the first step in the generation of new surface-based devices that have the capacity to be fully synthetic, selective, inexpensive and disposable.[6]

1. McIntyre C. *et al*., *Bioprocess International*, 2010, 44-53.

2. Chabert M. and Viovy J., *PNAS*, 2008, **105**, 3191-3196.

3. Shim S. *et al*., *Integrative Biology*, 2011, **3**, 850-862.

4. Kose A. R. *et al*., *PNAS*, 2009, **106**, 21478-21483.

5. Bulte J. W. M. *et al*., *Blood*, 2004, **104**, 3410-3413.

6. Singh A. *et al*., *Nature Methods*, 2013, **10**, 438-444

9:00am **BI+AS+IS+NL-MoM3 Interaction of Hematopoietic and Leukemic Cells with their Microenvironment**, ***A. Rosenhahn***, Ruhr-University Bochum, Germany, *M. Hanke, C. Christophis,* Karlsruhe Institute of Technology, Germany, *I. Taubert, N. Baran, P. Wuchter, A.D. Ho*, University of Heidelberg, Germany

Especially for leukemic and haematopoietic cells, the interaction with their microenvironment is of upmost importance for extravasation and homing. One key mechanism is the interaction of the CD44 receptor with extracellular hyaluronan (HA) binding motifs. To quantitatively assess the interaction, a microfluidic experiment has been developed that allows studying the interaction of cells with interfaces under well-defined flow conditions [1]. Shear flow activated catch bond interaction is well characterized for selectin mediated extravasation of leukocytes [2]. We recently found that also the CD44 interaction with HA requires a minimum shear stress to become activated and enable cells to roll on HA surfaces [3]. Similar critical shear values were found for rolling on mesenchymal stroma cells, which are present in the bone marrow niche creating the microenvironment required for haematopoietic stem cell renewal. Interestingly not only hematopoietic stem cells but also acute leukemic blasts show a shear flow induced rolling. The proportion of rolling cells will be discussed on the basis of the pathogenesis of the disease.

[1] C. Christophis, M. Grunze, A. Rosenhahn, PCCP 2010, 12, 4498.

[2] E.B. Finger, K.D. Puri, R. Alon, M.B. Lawrence, U.H. von Andrian, T.A. Springer, Nature 1996, 379, 266

[3] C. Christophis, I. Taubert, G. Meseck, M. Schubert, M. Grunze, A. D. Ho, A. Rosenhahn, Biophys. J. 2011, 101, 585.

9:20am **BI+AS+IS+NL-MoM4 The Creation of Polymeric Biointerfaces using Non-Contact Dispensing Technology**, ***C. Dufresne***, Scienion

Polymeric surfaces of varied composition have been created in high density microarray formats. These patterned surfaces have been used to study a number of biointerface processes such as stem cell differentiation, and bacterial adhesion. Scienion offers non-contact picoliter dispensing technology that enables the creation of such surfaces. The inert glass capillaries allow for the use of a wide range of chemical reagents. Precision positioning enables drop-on-drop dispensing and mixing. Image analysis of the substrates in turns makes it possible to accurately dispense the materials onto almost any surface. This presentation will cover how Scienion technology is implemented for the production of polymeric surfaces.

9:40am **BI+AS+IS+NL-MoM5 The Role of Cell-Substrate Interactions on Cell Stiffness and Cell Volume**, ***D.A. Weitz***, Harvard University **INVITED**

Cell stiffness is often observed correlate with the stiffness of the substrate on which the cells are grown. This talk will present data which suggest that cell-substrate interactions are more diverse, and depend as well on the adhesion area. It will discuss the impact of the substrate on cell volume and the consequences of this on cell stiffness. The data presented will suggest that cell volume is a control for cell stiffness.

10:40am **BI+AS+IS+NL-MoM8 Quantitative, Predictive Models of Adhesion of Cells to Polymers**, ***V.C. Epa***, *D.A. Winkler*, CSIRO Materials Science & Engineering, Australia, *A.L. Hook, C. Chang, J. Yang*, University of Nottingham, UK, *R. Langer, D.G. Anderson*, MIT, *P. Williams, M.C. Davies, M.R. Alexander*, University of Nottingham, UK

Designing materials to control biology is an intense focus of biomaterials and regenerative medicine research. Discovering and designing materials with appropriate biological compatibility or active control of cells, tissues, or pathogens is being increasingly undertaken using high throughput synthesis and assessment methods.

In particular, culture of multipotent cells such as stem cells is a major research focus in regenerative medicine. Much research effort is focused on designing chemically defined, serum-free, feeder-free synthetic substrates and media to support robust self-renewal of pluripotent cells. Changes in cellular properties such as adhesion, morphology, motility, gene expression and differentiation are influenced by surface properties of the materials on which cells have been cultured. Similarly, designing new materials to control the growth of pathogens on implantable and indwelling devices such as pacemakers, and catheters, is critical given the high level of device-centred infections.

We report a relatively simple but powerful machine-learning method of generating models that link microscopic or molecular properties of polymers or other materials to their biological effects. We illustrate the potential of these platform modelling methods by developing the first robust, predictive, quantitative, and purely computational models of adhesion of human embryonic stem cell embryoid bodies, and three clinically important pathogens, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and uropathogenic *Escherichia coli*, to the surfaces of 496 polymers.

11:00am **BI+AS+IS+NL-MoM9 Smart Surfaces for Studies of Real-Time Dynamic Cell Behavior**, ***M.N. Yousaf***, York University, Canada

Active migration, local tissue invasion and seeding of distant metastases are all characteristics of malignant cells. These complex cellular events require the integration of information derived from soluble growth factors with positional information gained from interactions with the extracellular matrix and with other cells. The biochemical events of the signaling cascades occur in a spatially and temporally coordinated manner that then dynamically shape the cytoskeleton in specific sub-cellular regions. Therefore cell migration and invasion involve a precise but constantly changing subcellular nano-architecture. To fully understand the complex signaling and cytoskeletal aspects of the cellular nano-architecture during migration requires a multidisciplinary coordinated effort. The long-term goal of this research program is to develop new surface chemistry and cell biological tools to generate a class of tailored dynamic nanopatterned substrates for a variety of cell adhesion and migration experiments. The combined application of dynamic smart substrates, molecular surface gradients and in vivo biosensors will potentially allow for the analysis and quantitation of the events of cell migration at each step from initial engagement with extracellular matrix ligands, to localized activation of signaling proteins, to organization and activation of the cytoskeleton, to overall movement of the cell.

11:20am **BI+AS+IS+NL-MoM10 What Makes the Heart Grow Fonder? Chemically Diverse Polyacrylate and Polyacrylamide Surfaces for Human Cardiomyocyte Culture and Their Effect on Phenotype**, ***A.K. Patel***, University of Nottingham, UK, *D.G. Anderson, R. Langer*, Massachusetts Institute of Technology, *M.C. Davies, M.R. Alexander, C. Denning*, University of Nottingham, UK

Human pluripotent stem cell (hPSC) derived cardiomyocytes hold the potential to strengthen pharmaceutical toxicity testing and to provide disease models for development of treatment targets1. The maturation and maintenance of the cardiomyocyte phenotype may be controlled by the manipulation of the substrate supporting the cells2. However, the surfaces currently in use still fall short of producing cardiomyocytes of adult maturity. Standard culture-ware requires coating with biological substrates such as fibronectin which can be expensive and subject to poor reproducibility due to batch variation. We are exploring an alternative, combinatorial materials high throughput screening approach3 to identify novel materials that can improve cardiomyocyte culture. Polymer microarrays comprising of 6 replicates of 116 acrylates and acrylamides are fabricated using contact printing. Cardiomyocytes derived from the HUES7 human stem cell line are seeded onto the arrays. Immunostaining of nuclei (DAPI) and the cardiomyocyte specific motor protein, sarcomeric alpha actinin is performed to visually estimate cell function and maturity and enable quantification of cell attachment in a high throughput manner using automated fluorescence microscopy and image analysis software. Surface characterisation of the arrays is performed using time of flight secondary ion mass spectrometry. Partial least squares (PLS) regression analysis allows for correlation of cell attachment with key molecular ions identified from mass spectrometry4.

Successful monomers that permit cardiomyocyte attachment, spreading and contraction are identified from the first generation homopolymer microarray and are mixed pair-wise to form second generation microarrays. This diverse library of copolymers enables unique combinations of chemical moieties to be investigated. Hit monomers and combinations identified to be synergistic can be analysed for their effect on cardiomyocyte function including electrophysiology measured by patch clamping, myofibril alignment and gene expression.

The lead materials generated by this approach are the first step in a discovery process for novel synthetic biomaterials capable of enhancing the culture of cardiomyocytes to move towards more reproducible, economical and defined conditions.

References:

1. Matsa E. et al. European Heart Journal. 2011;32(8):952-62

2. Engler A. et al.The Journal of Cell Biology. 2004;166(6):877–887

3. Hook A. et al. Biomaterials. 2010;31(2):187–198

4. Yang J. et al.Biomaterials. 2010;31(34): 8827–8838

11:40am **BI+AS+IS+NL-MoM11 Selectivity in Platelet Activation by the Titania Surface: A Model System for In Vitro Modulation of Platelet Activity**, *S. Gupta*, CIC biomaGUNE, Spain, ***I. Reviakine***, Karlsruhe Institute of Technology, Germany

Platelet are anuclear cell fragments circulating in blood. Their major function is haemostasis: they catalyze the formation of the fibrin clot that stops the bleeding. Recently it was shown that they have a multitude of other functions in processes such as the immune response, inflammation, angiogenesis, implant rejection or integration.

Platelets circulate in the blood in a quiescent form. They become activated at wound sites, implant surfaces, or through the action of soluble agonists secreted by activated platelets or produced in the blood as a result of the clotting process. Activated platelets express on their surface a variety of protein and lipid receptors that catalyze the clotting process, interact with other platelets, leukocytes, and endothelial cells, and adhere to the extracellular matrix exposed at the wound sites. The also secrete a variety of active substances, including growth factors, that are stored inside special granules within the platelets.

Recently discovered diversity of platelet functions implies a tight regulation of the activation processes. Indeed, there is evidence to suggest that platelet activation is a selective process with a spectrum of activated states, rather than a two-state process involving quiescent vs. pro-coagulant platelets. In this context, we have previously shown that platelet activation profile on TiO_2 depends on the surface-bound Ca. Here, we measure intracellular calcium currents in surface-adsorbed platelets in order to understand how this manifestation of platelet activation selectivity is related to the internal signaling pathways. Such an understanding is a prerequisite for designing new, platelet-based approaches to the treatment of haemostatic and inflammation-based disorders, to enhancing implant integration and wound repair, and to tissue engineering applications.

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+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.

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.

Applied Surface Science
Room: 204 - Session AS+BI+EM+NL+NS+SS-ThM

Nanoparticle Surface Chemistry
Moderator: H. Zuilhof, Wageningen University, Netherlands, D.Y. Petrovykh, International Iberian Nanotechnology Laboratory, Portugal

8:00am **AS+BI+EM+NL+NS+SS-ThM1 Surface Analysis as a Critical Step in Translating Nanomaterials to Technologies**, ***D.W. Grainger***, University of Utah **INVITED**

Difficulties assessing human exposure, safety, and possible toxicity from nanotechnologies have prompted questions about how to characterize nanomaterials in various experimental test beds for predictive use. While little consensus is published about human risk/benefit analysis, this is confounded by lack of accepted, sensitive and reliable characterization methods of practical value for nanomaterials in physiological milieu. Relatively few studies are conducted on these materials in biologically relevant media to understand their surface properties and physical states (i.e., sedimentation, aggregation) prior to in vitro or in vivo exposures. Few studies have standard reference materials and analytical protocols established for comparisons to other studies. The current understanding of the fate of nanomaterials of most any size and shape, both in cell culture mediawith serum or inside the mammalian body, is poor at best. Additionally, the collective published scientific record documenting fate of nanomaterials in vivo is consistent with long known tissue-based particle filtration for micro-colloids, with far less success deliberately targeting particles to specific tissue or disease sites (i.e., <5% of a nanoparticle dose reaches a disease site).

To date, most data suggest that size reductions to the nanometer dimension have not significantly changed how nanomaterials interact with physiological systems in vivo, despite in vitro distinctions observed with proteins and in cell cultures. Connecting nanomaterials properties with how they interact with proteins and cells in vitro to affect their biodistribution in vivo allows a more rational approach to designing nanomaterials with specific biomedical and toxicity properties, and to avoid the ubiquitous non-specific tissue scavenging. This is related to materials interactions with whole blood components, including platelets, cells, and plasma proteins, producing fluid transport to tissue sites, particle binding, opsonization and aggregation. However, analytical methods for nanomaterialsare not sufficiently sensitive to study these effects in vivo to alter nanomaterials biodistribution patterns. Additionally, understanding how surface coatings, ligands and contaminants change physiological behavior requires careful analysis.

The nanotechnology field must develop improved, sensitive analytical tools and methods to drive a consensus for how nanomaterials (1) should be fully and reliably characterized for biological and biomedical purposes, and (2) how different nanomaterials properties produce either beneficial (i.e., therapeutic) or toxic responses inside complex physiological systems.

8:40am **AS+BI+EM+NL+NS+SS-ThM3 Molecular Surface Characterization of Individual Nano-objects**, *C.-K. Liang, S.V. Verkhoturov,* ***E.A. Schweikert***, Texas A&M University

The importance of surface characterization of nano-objects in dimensions below 50 nm is well recognized. Indeed the most pronounced changes in chemical reactivity are expected to occur on the smallest size nano-objects. Yet, as their size shrinks, measurement techniques are lagging. A further concern, when seeking insight into size-composition-reactivity relationships, is population heterogeneity. We present here a technique for assaying individual nano-objects. The method involves bombarding dispersed nano-objects one-by-one with nanoprojectiles specifically Au_{400}^{4+}, at hypervelocity. Their impact causes abundant emission of ionized ejecta which are identified individually for each impact by time-of-flight mass spectrometry. We will describe the characterization of surfaces and environments of nano-objects as revealed by selected grazing projectile impacts.

9:00am **AS+BI+EM+NL+NS+SS-ThM4 Towards the Effective Combination of Static and Dynamic SIMS for Nanoparticle and Biological Analyses**, ***C. Szakal***, National Institute of Standards and Technology

Static SIMS and dynamic SIMS experiments, protocols, instrumentation, and laboratory groups have largely developed in separate paths. As a result, it is common to think of certain application areas for a specific SIMS analysis, such as semiconductor depth profiling with dynamic SIMS and molecule-specific imaging with static SIMS. However, a combination of the two SIMS methodologies could generate a more complete data set by utilizing the surface-sensitive characteristics of ToF-SIMS with the enhanced signal dynamic range of large geometry (LG) dynamic SIMS. Benefits and potential pitfalls of such a combined analysis are discussed for nanoparticle surface chemistry vs. bulk measurements along with other biological application areas.

9:20am **AS+BI+EM+NL+NS+SS-ThM5 Quantitation of Protein Adsorption to Gold Nanoparticles**, ***C. Minelli,*** *N.C. Bell, A.G. Shard*, National Physical Laboratory, UK

The ability to quantitatively describe protein coronas of nanoparticles in biological fluids is highly sought for to understand protein corona formation, nanoparticle fate and their interaction with biological systems. A quantitative description of the nanoparticle biomolecular interface is challenging and chemical information along with structural and biofunctional characterization requires the use of complementary techniques.

The parallel use of different techniques provides in fact a range of complementary information of the materials under study, each technique being based on a specific physical principle. Here, we combine the use of liquid-based size measurement techniques such as Dynamic Light Scattering (DLS), Nanoparticle Tracking Analysis (NTA) and Differential Centrifugal Sedimentation (DCS), with vacuum techniques such as X-ray Photoelectron Spectroscopy (XPS) to provide quantitative information of core/shell nanoparticle systems.

We used a set of spherical gold nanoparticles having diameters from 20 nm to 80 nm and coated with different amount of Immunoglobulin G (IgG) antibodies as a model system. The shift of the nanoparticle Localized Surface Plasmon Resonance (LSPR) frequency measured by UV-Vis spectroscopy is known to relate to the amount of molecules adsorbed at nanoparticles' interface. We measured the LSPR shifts along with the nanoparticle sizes in liquid by using DLS, NTA and DCS. When the nanoparticles had a complete protein shell, shell thickness measurements were consistent for all the techniques. DCS sedimentation times showed excellent correlation with LSPR frequency shifts, indicating that analytical centrifugation can provide precise measurement of the thickness of complete protein shells on nanoparticles. However, in the low coverage regime, NTA and DLS techniques provided the best correlation with LSPR frequency shifts. By combining the information from the different techniques we estimated the amount of IgG molecules per nanoparticle as a function of the IgG concentration in solution. Data analysis in the high concentration regime suggested that nanoparticle curvature strongly influences the ability of a surface to allow the further adsorption of IgG.

Core/shell nanoparticle systems were also characterized by XPS. A methodology for the preparation of nanoparticle samples for analysis in vacuum was developed. XPS data was collected from nanoparticles of different core size and analysis provided quantitative chemical information of the nanoparticle. The combined use of XPS with liquid-based techniques for particle characterization provided quantitative chemical and structural information of core/shell nanoparticle systems.

9:40am **AS+BI+EM+NL+NS+SS-ThM6 Surface Characterization of Protein Functionalized Gold Nanoparticles**, *Y.-C. Wang, A. Rafati,* ***D.G. Castner***, University of Washington

Nanoparticles exhibit unique surface properties and require well-controlled surface properties to achieve optimum performance in complex biological or physiological fluids. Thus, there is a need to develop rigorous and detailed surface analysis methods for their characterization. The surface chemistries of oligo(ethylene glycol) (OEG) self-assembled monolayers (SAMs) on Au nanoparticle (AuNP) surfaces were characterized with x-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), Fourier transform IR spectroscopy and high-sensitivity, low-energy ion scattering (HS-LEIS). The size, shape, and size distribution of the AuNPs was determined by transmission electron microscopy (TEM).

Both methoxy (CH_3O-) and hydroxyl (HO-) terminated OEG SAMs with chains containing 11 methylene and 4 ethylene glycol units were examined. ToF-SIMS clearly differentiates the two OEG SAMs based on the $C_3H_7O^+$ peak attributed to the CH_3 terminated SAM, while XPS didn't detect a significant difference between the two SAMs on the same surface. However, XPS did show a significant difference between the same SAM on different sized AuNPs. Both OEG SAMs were more densely packed on the 40 nm diameter AuNPs compared to the 14 nm diameter AuNPs. FTIR experiments indicates the methylene backbone groups are well-ordered on

all gold surfaces, but the OEG groups are more ordered on the 40 nm diameter AuNPs. Together the XPS and FTIR results suggest the OEG SAMs form a thicker and/or higher density SAMs on the 40 nm AuNPs compared to the 14nm AuNPs. HS-LEIS experiments showed the OEG SAMs on the 40 nm AuNPs were significantly thicker (2.6 nm) than the OEG SAMs on the 14 nm AuNPs (2.0 nm) and the flat Au surface (1.9 nm). The 2.6 nm thickness measured on the 40 nm AuNPs is consistent with thickness expected for a well-order OEG SAM (2.7 nm). TEM showed the 40 nm AuNPs had a larger size distribution and were less spherical compared to the 14 nm AuNPs, suggesting the shape of the AuNPs can have a significant effect on the structure and thickness of the OEG SAMs.

Protein G was immobilized onto the HO-terminated OEG SAMs via carbonyl diimidazole chemistry. ToF-SIMS analysis showed the relative intensities of characteristic amino acid fragments from Protein G varied with both the protein solution concentration and the type of surface.

10:40am **AS+BI+EM+NL+NS+SS-ThM9 Optical Rotation Measurements of Enantioselective Separation on Chiral Au Nanoparticles**, ***N. Shukla***, *N. Ondeck, N. Khosla, A.J. Gellman*, Carnegie Mellon University

Adsorption of chiral compounds on chiral surfaces is the initial step in enantioselective processes such as separations and catalysis. There has been a significant effort over the past decade aimed at the preparation of chiral nanoparticles based on metallic cores modified by chiral ligands. In principle, these can serve as the basis for enantioselective chemical processing. In this work we demonstrate a simple measurement of enantioselective adsorption on chiral metal nanoparticles using a method that can yield quantitative measures of the enantiospecific adsorption equilibrium constants [1].

The surfaces of chemically synthesized Au nanoparticles have been modified with D- or L-cysteine to render them chiral and enantioselective for adsorption of chiral molecules. Their enantioselective interaction with chiral compounds has been probed by optical rotation measurements when exposed to racemic propylene oxide. The ability of optical rotation to detect enantiospecific adsorption arises from the fact that the specific rotation of polarized light by R- and S-propylene oxide is enhanced by interaction Au nanoparticles. This effect is related to previous observations of enhanced circular dichroism by Au nanoparticles modified by chiral adsorbates. More importantly, chiral Au nanoparticles modified with either D- or L-cysteine selectively adsorb one enantiomer of propylene oxide from a solution of racemic propylene oxide, thus leaving an enantiomeric excess in the solution phase. Au nanoparticles modified with L-cysteine (D-cysteine) selectively adsorb the R-propylene oxide (S-propylene oxide). A robust model based on optical rotation data has been developed that allows extraction of the enantiospecific equilibrium constants for R- and S-PO adsorption on the chiral Au nanoparticles.

[1] N. Shukla, M.A. Bartel, A.J. Gellman "Enantioselective separation on chiral Au nanoparticles" Journal of the American Chemical Society, 132(25), (2010), 8575–8580

11:00am **AS+BI+EM+NL+NS+SS-ThM10 Monitoring the Citric Acid Content on Dialyzed Gold Nanoparticle**, *V. Spampinato, R. La Spina, D. Gilliland, L. Calzolai*, ***G. Ceccone***, *F. Rossi*, EC-JRC-IHCP, Italy

Gold nanoparticles (GNPs) are probably the most investigated metal nanomaterials due to their interesting properties. In fact, GNPs are applied in a several areas including material sciences, catalysis and biomedical diagnostics[1] Most of the applications of GNPs in the medical and biosensing fields require the development of careful purification to obtain afterwards a more efficient surface functionalization.[3,4] The process of purification is usually obtained by filtration, centrifugation and/or dialysis of the GNPs solution to remove part of the citrate or other stabilizing agents.[5,6] The citrate reduction Au(III) in water, known as Turkevich method, is one of the most used synthesis process to produce monodispersed and stable GNPs. [8] In this synthesis, the citrate is either the reducing agent and the stabilizer and it is used in large excess in comparison to the amount of gold.[9] In this work, we have investigated the stability and effect of dialysis on citrate stabilized GNPs by quantifying the content of citrate by Nuclear Magnetic Resonance (^{1}H-NMR), and by characterizing the GNPs/citrate interface chemistry using X-ray Photoelectron Spectroscopy (XPS) and Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS). In these studies, 15 nm gold nanoparticles stabilized with the citrate have been synthesized via the Turkevich process and the content of citrate was monitored at several or different cycles of dialysis against ultrapure water. These systematic studies showed a decreasing of the citrate content with the dialysis cycles. In particular, XPS and ToF-SIMS show that for low dialysis cycles the ratio Au/Na and Au/CHO increase almost linearly, while after 9 dialysis cycles a plateau is reached. A Similar trend is observed by^{1}H-NMR where the amount of citrate is quantified against an internal standard. The behavior of the GNPs at different dialysis cycles was also monitored by Centrifuge Particle Separation (CPS) and Dynamic Light Scattering (DLS) and UV-Vis spectra, revealing that several dialysis cycle result in a partial aggregation of the nanoparticles.

[1] P-J Debouttière, et al., *Adv. Funct. Mater.*, **2006**, *16*, 2330

[3] N. L. Rosi, et al., *Science*, **2006**, 312, 1027

[4] C. Murphy et al., *Acc. Chem. Res.*, **2008**, *41* (12), 1721

[5] S. Techane, et al., *J Phys Chem C*, **2011,** 115(19), 9432

[6] S. Sweeney et al., *J. Am. Chem. Soc.*, **2006**, *128* (10), 3190

[7] C. Chen, et al.*Nano Lett.*, **2006**, *6* (4), pp 611–615

[8] J. Turkevich, et al., *Discuss. Faraday Soc.*, **1951**, 11, 55

[9] M. Doyen, et al, *J. Coll. Int. Sci.*, **2013,** 399, 1

11:20am **AS+BI+EM+NL+NS+SS-ThM11 Comparison of the Structure and Solution Behaviors of 20nm Silver and 20nm Silver-Shell-Gold-Core Nanocomposites in Aqueous Biological Media**, *P. Munusamy,* Pacific Northwest National Laboratory, *S. Chen, L.B. Yen,* Imperial College London, UK, *C.W. Wang, M. Engelhard,* Pacific Northwest National Laboratory, *A. Porter,* Imperial College London, UK, ***D.R. Donald***, Pacific Northwest National Laboratory

Different synthesis routes have made it possible to produce silver nanoparticles with variety of structure properties such as size, shape, and surface functionality. Synthesizing or processing silver nanoparticles under different conditions can impart variations in properties and reactivity which can influence the biological end points. In this study we have examined how silver nanoparticles of nearly identical size, as measured by dynamic light scattering, but produced with and without gold core behave in the biological media RPMI 1640 with FBS, the cell culture media used in our laboratory for in vitro nanotoxicity studies. The initial physico-chemical characterization of citrate capped nanoparticles using DLS size and surface charge measurement showed particles of average size 27nm with negative surface charge. Structure and compositional analysis using STEM and XPS confirmed the presence of gold core of size ~7nm in one set of particles. The detailed structure of the pure silver and the core-shell particles differ significantly. Based on TEM images and XRD measurements, the pure silver particles are highly crystalline, made up of ~ 15-20 nm crystallites with well-defined grain boundary or slip plane defect structures. The silver surrounding the gold core is made up of smaller highly disordered crystallites. After 24h incubation in culture media, STEM images showed that the particles with Au core dissolved significantly and non-uniformly indicating solution attack down to the gold core. In contrast, pure silver particles underwent more uniform dissolution with some indication of varying rates for different crystal faces. In addition to the dissolution of the primary particles of both types, new smaller "daughter" silver particles were observed both nearby or some distance away from the initial nanoparticles. Centrifugation followed by ICP-MS analysis of the supernatant was used to quantify the amount of dissolved silver. The dissolution extent for core-shell particles in 24h was 3 times higher than that for pure Ag. These results highlight the significance of synthesis route and sample structure on the solution behavior of similar nanoparticles in biologically relevant environmental conditions.

11:40am **AS+BI+EM+NL+NS+SS-ThM12 Influence of Carrier Gas on the Nucleation and Growth of Nb Nanoclusters Formed Through Plasma Gas Condensation**, ***K.R. Bray***, *C.Q. Jiao,* UES, Inc., *J.N. DeCerbo*, Air Force Research Laboratory

The synthesis and characterization of metallic nanoclusters is a growing field of research due to their promising catalytic, electrical, magnetic, mechanical, and optical properties. These properties generally differ from the bulk material and can be tuned by varying the nanocluster size. Transition metal clusters have received considerable interest due to their wide range of applications. Niobium has attracted attention due to observations of ferroelectric properties at low temperature. In this work, Nb nanoclusters are deposited using a plasma gas condensation process which involves the sputtering of a Nb target to create a dense metallic vapor where clusters are formed. The concept of a temperature dependent nucleation zone in conjunction with classical nucleation theory is used to describe nanocluster nucleation and growth. Changes in the nanocluster nucleation and growth are influenced through modifications of the process parameters such as carrier gas flow rate, sputter source ion current, and aggregation length. Initial data show a novel dual peak cluster distribution under select process conditions, with the smaller cluster diameter near 1 nm and the larger cluster diameter varying from 4 to 10 nm. The larger cluster appears to be a simple condensation product while data suggest the smaller cluster may be a structured cluster with a different nucleation and growth mechanism. The effects of differing argon and helium carrier gas ratios on cluster formation in conjunction with varying sputter source currents and aggregation lengths will be discussed. These results provide the opportunity for a broader understanding into the nucleation and growth of nanoclusters

as well as insights into how process parameters interact during deposition. This knowledge will enhance the ability to create nanoclusters with desired size dispersions.

Biomaterial Interfaces
Room: 201 B - Session BI+NL+NS+SS-ThM

Bio/Nano Interfaces
Moderator: G.J. Leggett, University of Sheffield

8:20am **BI+NL+NS+SS-ThM2 Utility of Lipid Membranes Assembled on Nanoparticles for Measuring Protein Membrane Interactions**, ***S.M. Reed***, University of Colorado Denver

Nanoparticles (NPs) provide a well-defined template for preparing supported lipid membranes with controlled curvature. We have coated supported lipid bilayers and hybrid membranes on silica and gold nanoparticles. The localized surface plasmon resonance (LSPR) of gold NPs can be used to monitor the assembly of lipid layers on NPs and to monitor protein lipid interactions. The gold LSPR is very sensitive to the immediate surroundings of the nanoparticle surface and therefore provides a method to monitor the coating of lipids and subsequent conversion of a supported bilayer to a hybrid membrane after the addition of hydrophobic alkanethiols. We demonstrate that both long chain (decanethiol) and short chain (propanethiol) anchors are able to form hybrid membranes and that these membranes allow for LSPR based detection of protein binding events at the membrane surface.

While many materials have been used as membrane supports, there are unmet needs in the development of membrane mimics and it remains challenging to monitor the coating process and to control the curvature of a membrane. Recent work has demonstrated that quantum dots, silica NPs, and gold NPs can be used as templates for membranes providing an opportunity to control curvature. Here, we have exploited the local refractive index sensitivity of the gold LSPR to observe the process of lipid-coating, structural rearrangement of supported membranes into hybrid membranes, and finally the binding of protein. The introduction of phosphatidylcholine (PC) to the gold NPs results in a rapid binding evidenced by a change in the wavelength of the LSPR, however, the interaction with gold is weak and the gold is not completely covered by the lipid. By adding a hydrophobic alkanethiol anchoring group, the lipids bind closer to the gold NP surface resulting in increased stability. This stability is achieved at different concentrations for short and long hydrophobic chains. When propanethiol was used it was possible to destabilize and remove the lipid coating by adding the hydrophilic thiol, beta-mercaptoethanol. This loss of membrane is observed through changes to the LSPR and increased permeability of the membrane to ions.

8:40am **BI+NL+NS+SS-ThM3 Primitive Osmosensing by Phospholipid Membranes**, *K. Oglecka,* Nanyang Technological University, Singapore, *J. Sanborn, D. Gettel,* University of California, Davis, *R. Kraut, B. Liedberg,* Nanyang Technological University, Singapore, ***A.N. Parikh***, University of California, Davis **INVITED**

This talk describes experimental observations of the response of multicomponent vesicles to osmotic gradients. We find that giant vesicles, consisting of phase separating lipid mixtures, immersed in hypertonic bath exhibit a Rayleigh-Plateu like pearling instability paving for elemental mechanical process of vesicular self-reproduction. When immersed in hypotonic bath, however, the response of giant vesicles comprise an unusual transitory state, characterized by damped, periodic oscillations between a microscopically phase-separated state and a uniform one. We find that this unusual oscillatory phase separation is synchronized with the cyclical patterns of membrane tension and poration, producing swell-burst cycles. Swelling, which is caused by the influx of water, raises membrane tension, thus promoting the appearance of microscopic domains. Bursting, which facilitates solute leakage, relaxes the membrane tension, breaking up large domains into those below the optical limit. This autonomous self-regulatory response – in which an external osmotic perturbation is managed by a co-ordinated and cyclical sequence of simple physical mechanisms. These mechanisms allow vesicles to sense (by domain formation) and regulate (by solute efflux) osmotic differences across their compartmental boundaries in a negative feedback loop producing a primitive form of a quasi-homeostatic regulation in a synthetic system, generated from simple components, namely, water, osmolytes, and lipids.

9:20am **BI+NL+NS+SS-ThM5 Ultrathin Poly(ethylene glycol) Films as Flexible Platform for Plasmonics and Lithography and as Precursors for Free-Standing Nanomembranes**, ***N. Meyerbröker***, *M. Zharnikov*, University of Heidelberg, Germany

We present a novel approach to prepare ultrathin, biocompatible films based on cross-linking of multi-functionalized, star-branched poly(ethylene glycols) (STAR-PEGs) with tunable film thicknesses of 4 – 200 nm. A two-component mixture of amine- and epoxy-terminated four-arm STAR-PEGs was spin-coated on a flat substrate and cross-linked chemically by gentle heating, resulting in a stable, hydrogel-like film with a density close to that of bulk PEG material. The films revealed pronounced swelling behavior, which was fully reversible and could be precisely controlled. Additionally, they provided a high affinity to citrate-stabilized gold nanoparticles (AuNP) that could be adsorbed with high densities into the PEG matrix from an aqueous solution. These novel PEG/AuNP composite films offer interesting and potentially useful optical properties. Controlling the accessibility, swelling behavior, and biorepulsive properties of the PEG films lithographically, we prepared nanocomposite patterns of metal nanoparticles and fluorophores imbedded into the PEG matrix as well as protein-affinity patterns in protein-repelling background. Further, using electron beam lithography, we succeeded to fabricate wettability patterns and to sculpture complex 3D microstructures on the PEG basis. Finally, we demonstrated that the PEG films can be separated from the substrate and exist as ultrathin, biocompatible, free-standing membranes. These membranes possess high stability and exceptional elasticity. They can be used in transmission electron microscopy experiments on sensitive biological targets and as a new type of support for the characterization of nanoparticles.

9:40am **BI+NL+NS+SS-ThM6 Miniaturized Localized Surface Plasmon Resonsance Sensing with Single Nanoparticle Arrays**, ***S. Chen***, *M. Svedendhal, T. Antosiewicz, M. Käll*, Chalmers University of Technology, Sweden

Ultrasensitive biosensing is one of the main driving forces behind the dynamic research field of plasmonics. I will show that the sensitivity of single metal nanoparticle plasmon spectroscopy can be greatly enhanced by enzymatic amplification of the refractive index footprint of individual protein molecules, so called plasmon-enhanced ELISA. The technique, which is based on generation of an optically dense precipitate catalyzed by horseradish peroxidase at the metal surface, allowed for colorimetric analysis of ultralow molecular surface coverages with a limit of detection approaching the single molecule limit. In addition I will show that by combining large arrays of well-separated gold nanoparticles fabricated by electron beam lithography (EBL) with hyper spectral imaging, spectral responses of up to 700 LSPR particles can be simultaneously studied. This allows us to obtain enough statistical significant number of spectra to further study the inhomogeneous broadening of the sensing properties of individual particles. This includes how variation in electric field enhancement over the surface of a single particle and variation in size and morphology of the enzymatic precipitate could affect the uncertainty in determining the number of enzyme molecules per particle. By combining the electromagnetic simulations with the measurements we could conclude that main sources of uncertainty come from variations in sensitivity across the surface of individual particles and between different particles. There is also a considerable uncertainty in the actual precipitate morphology produced by individual enzyme molecules. I will also discuss the possible improvement that can be done to achieve digital responses from the enzymatic amplified single particle sensing.

10:40am **BI+NL+NS+SS-ThM9 Design of Nanoscale Bionterfaces by Self-Assembly of Genetically Encoded Peptide Polymers**, ***A. Chilkoti***, Duke University **INVITED**

This talk will cover work in my laboratory over the past decade on the self-assembly of genetically encoded stimulus responsive elastin-like polypeptides (ELPs). We have exploited ELPs to create stimulus responsive nanostructures via three approaches. In the first approach, we have designed diblock ELPs with two ELP blocks with different hydrophobicity's that self-assemble into spherical micelles with an increase in temperature about the critical micellization temperature of the diblock polymer. Building on this architecture, we have incorporated histidine residues in the hydrophobic block to create a diblock ELP that self-assembles into spherical micelles with an increase in temperature, while a small drop in pH from 7.4 to 6.4 leads to micelle disassembly. In a second –chemical attachment triggered self-assembly– approach, we have shown that the attachment of multiple copies of small molecule hydrophobes to the multiple cysteine (C) residues of an ELP with the sequence $(VPGXG)_n(CGG)_8$ can drive their self-assembly into spherical micelles. In a third approach, we replace the Cys (C) with W, Y, or F, and find that oddly, this leads to the formation of stimulus responsive worms and vesicles depending on the specific residue.

These are the first examples of stimulus responsive worms and vesicles in peptide polymers.

11:20am **BI+NL+NS+SS-ThM11 Label-Free Mapping of Protein/Peptide Interactions in Complex Arrays Using Core/Shell Nanoparticle-Based Biosensors**, *H.O. Guvenc*, University of Heidelberg, Germany, *C. Schirwitz, F. Breitling*, Karlsruhe Institute of Technology, Germany, *F.R. Bischoff*, German Cancer Research Center Heidelberg, Germany, *A. Nesterov-Mueller*, Karlsruhe Institute of Technology, Germany, *V. Stadler*, PEPperPRINT GmbH, Heidelberg, Germany, *J. Wagner*, ***R. Dahint***, University of Heidelberg, Germany

The detailed analysis of biospecific interactions is of crucial importance in biomedicine, biotechnology, and pharmacology.Important applications range from medical diagnosis and drug development to the screening of the human genome and proteome. In recent years, array concepts have become very popular and powerful tools to allow for highly parallel, rapid identification of binding events. These arrays contain a multitude of different probe molecules immobilized at specific locations of an underlying substrate.

Interaction analysis is usually facilitated by labelling the potential binding partners with additional markers. Today, many of such techniques are well-established yielding considerably low detection limits and high lateral resolution. Yet, they suffer from the fact that labelling procedures are usually costly and time-consuming, that the labelling efficiency needs to be properly controlled for quantitative analysis, and that the marker itself can affect the original functionality of the molecules being studied. Moreover, the detection of low-affinity binding events is often hampered as additional washing steps and (bio)chemical reactions are required in-between the interaction and detection processes. To overcome those obstacles, strong efforts have been made to establish label-free detection schemes for interaction analysis. However, marker-free, sensitive readout of high-density arrays is still a technological challenge.

Here we report recent experiments on the label-free detection of protein/peptide interactions in complex arrays based on surface plasmon imaging with core-shell nanoparticle monolayers. Upon reflection of white light, these films exhibit a pronounced extinction spectrum which shifts to higher wavelength upon molecule binding, thus, providing a simple, sensitive and label-free detection mechanism. Variants of HA (human influenza hemagglutinin A) and FLAG epitopes with permuted amino acid sequence are synthesized in array format by means of combinatorial chemistry using a novel laser printing approach. After array preparation, the pattern is cleaved from the carrier and transferred to a biosensor surface consisting of core-shell nanoparticle films. By this means, the arrays are purified from synthesis artefacts caused by incomplete coupling reactions in the synthesis without the loss of spatial resolution. The interaction of the different peptides with their respective antibodies is quantified by the wavelength shift observed for individual peptide spots and compared to fluorescence-based interaction analysis. Based on the data we conclude on relevant amino acid sequences for an efficient antibody/epitope binding.

11:40am **BI+NL+NS+SS-ThM12 Graphene for Biosensing and Surface Functionalization**, ***P.E. Sheehan***, Naval Research Laboratory, *R. Stine*, Nova Research, *S.P. Mulvaney, J.T. Robinson, C.R. Tamanaha*, Naval Research Laboratory

Graphene, a one-atom thick sheet of sp^2 carbon, offers many intriguing possibilities in the field of molecular sensing. Its unique combination of large areas with nanometer thickness and high electrical conductivity could enable small scale device sensitivity with large scale production methods. A major benefit of using graphene is the large toolbox of well-established chemistries for incorporating chemical functionalities or specific recognition elements at the device surface. Here, we will discuss our efforts to develop graphene-based biological field-effect transistors (BioFETs), which offer sensitivity comparable to sensors made with other nanoscale materials (carbon nanotubes, nanowires), but with greatly simplified production methods common in the semiconductor industry. Devices utilizing both graphene and graphene oxide will be covered, and surface spectroscopic studies of the material modification will be discussed. Successful results for the detection of specific DNA hybridization using graphene BioFETs will also be presented. We will further discuss our efforts to use graphene as a biofunctionalized interface for a number of materials, from polymers to dielectrics to semiconductors, of interest to the biosensing community. Graphene's ultrathin nature allows its inclusion in more traditional sensing platforms as a non-intrusive functionalization layer, discreetly lending its chemical flexibility to other, more inert materials without significantly impacting the sensing device.

Applied Surface Science
Room: 204 - Session AS+BI+EM+NL+NS+SS-ThA

Nanoparticle Surface Chemistry II
Moderator: N. Kruse, Université libre de Bruxelles, Belgium

2:00pm **AS+BI+EM+NL+NS+SS-ThA1 Fundamental Explorations of Chemical Bonding and Surface Chemistry at Graphene Interfaces**, *B.J. Schultz, V. Lee, R. Dennis, J. Aldinger, S. Henderson,* ***S. Banerjee***, University at Buffalo, The State University of New York **INVITED**

The distinctive 2D sp^2-hybridized structural framework of graphene gives rise to a unique electronic structure characterized by conical valence and conduction bands touching at the Dirac point with linear energy dispersion within ± 1 eV of the Fermi level. Given the entirely surficial geometric structure of graphene, the extent of manifestation of true Dirac physics in this material is substantially modulated by perturbations of the electronic structure as a result of interactions with charged impurities, coupling to the underlying substrate, orbital hybridization with deposited contacts, and buckling/corrugation of graphene sheets. I will focus on the results of our combined X-ray absorption spectroscopy, Raman microprobe analysis, and density functional theory studies of graphene/metal and graphene/dielectric interfaces. Depending on the nature of the transition metal and the proximity of the graphene surface, physisorption or covalent chemical bonding is observed. Studies of the hybridization of single-crystalline metal surfaces with graphene suggest clear facet selectivity. We further evidence the potential for anisotropically functionalizing only one surface of planar graphene. For dielectric interfaces, charge transfer is observed without formation of carbidic bonds. Next, I will discuss our recent results on nitrogen incorporation within graphene oxide achieved through chemical reduction or annealing under a NH_3 atmosphere. Using near-edge X-ray absorption fine structure spectroscopy in conjunction with electrical transport measurements, we have developed a detailed picture of the recovery of the electronic structure of graphene oxide upon chemical or thermal defunctionalization. I will further discuss the design of graphene—polyetherimide nanocomposites based on engineered graphene interfaces that endow remarkable corrosion protection to low alloy steel upon application as thin films.

2:40pm **AS+BI+EM+NL+NS+SS-ThA3 Structure-dependent Trends in Adsorption of CO, O_2, and H_2 on Pd and Pt Nanoparticle Catalysts**, ***H. Mistry***, *F. Behafarid, B. Roldan Cuenya*, University of Central Florida

Many important catalytic reactions have shown striking dependence on particle size and shape. Therefore, understanding structure-dependent adsorption processes using model nanoparticles is key to designing highly active and selective catalysts. Temperature-programmed desorption and x-ray absorption fine structure spectroscopy were used to study the interaction of adsorbates with Pt and Pd nanocatalyts. The binding strength of oxygen and carbon monoxide adsorbed on Pd nanoparticles supported on SiO_2/Si(111) was shown to increase with decreasing particle size. In addition, pressure-dependent changes in hydrogen coverage and structure of size- and shape- selected Pt/γ-Al_2O_3 nanoparticles were investigated.

3:00pm **AS+BI+EM+NL+NS+SS-ThA4 CO-induced Scavenging of Oxide-Supported Platinum Nanoclusters**, *N. Chaabane,* INSTN, CEA, France, *R. Lazzari,* ***J. Jupille***, INSP, UPMC and CNRS, France, *G. Renaud,* INSC, CEA, France, *EA. Soares*, ICEx-IFMG, BeloHorizonte MG, Brazil

The efficiency of oxide-supported catalysts frequently relies on the dispersion of the metallic particles whereas the optimization of the proportion of active atoms of the often precious metals involved in catalysts is an economic issue. Beyond the achievement via synthesis processes of the optimum morphology that accounts for the combination of those constraints, a great attention is paid to the phenomena which drive changes in shape, size and structure of the clusters of catalysts in running conditions. Aside the capability to resist high temperature aging, a main concern is the sustainability of catalyst particles upon exposure to reactive atmospheres. A prototypical case is the effect of CO on transition metals catalysts, of which supported platinum is a thoroughly studied example because it combines a strong practical relevance with puzzling stability behavior in the presence of CO [1]. Under CO exposure, disruption and agglomeration of supported Pt clusters were simultaneously evidenced by extended x-ray absorption fine structures, scanning tunneling microscopy and infrared spectroscopy. However, those parallel phenomena are not explained yet.

In the present work, changes in size and shape of MgO(100)-supported Pt nanoclusters were tracked *in situ* by Grazing Incidence Small-Angle X-Ray scattering (GISAXS) at CO pressures ranging from 10^{-6} to 10^{3} Pa [2]. MgO has been chosen as an archetype of non-reducible support giving rise to abrupt interfaces with platinum [3]. Between 300 K and 470 K, Pt particles smaller than a critical size of 1 nm were shown to disrupt at CO pressure as low as 10^{-1} Pa. Once formed, the disrupted particles - suggested to be carbonyl moieties - underwent scavenging by clusters larger than the critical size. Disruption and agglomeration are both consistent with a CO-driven ripening mechanism [4]. An additional agglomeration mechanism was evidenced. Upon annealing up to the desorption temperature of CO, CO-covered Pt clusters of size ranging between the critical value and 2 nm were seen to agglomerate by diffusion; this is discussed in terms of an adsorbate-induced weakening of the cluster-support bonding. Similar CO-induced mechanisms (ripening and cluster diffusion) are suggested to hold for other supported metal catalysts such as Ru, Rh and Ir.

[1] Y. Nagai et al., Catal. Today 175 (2011) 133.

[2] N. Chaâbane, R. Lazzari, J. Jupille, G. Renaud and E.A. Soares, J. Phys. Chem. C 116 (2012) 23362.

[3] J. Olander, R. Lazzari, J. Jupille, B. Mangili, J. Goniakowski and G. Renaud, Phys. Rev. B 76 (2007) 075409.

[4] R. Ouyang, J.-X. Liu and W.-X. Li, J. Am. Chem. Soc 135 (2013) 1760.

3:40pm **AS+BI+EM+NL+NS+SS-ThA6 Adsorption Energies of Cu Nanoparticles on CeO_{2-x}(111) Supports Studied by Microcalorimetry**, ***T.E. James***, *S.L. Hemmingson, C.T. Campbell*, University of Washington

The increasing demand for energy has accelerated the need to develop new and improved catalysts for existing and alternative technologies. Heterogeneous catalysts consisting of transition metal nanoparticles dispersed across oxide supports are found in solar cells, fuel cells, industrial chemical production and environmental cleanup. Fundamental understanding of these supported catalysts, such as the bond energies between the metal clusters and their supports, which is crucial to understand the sintering behavior and catalytic reactivity, is still largely missing. This work uses Cu clusters and a single-crystal ceria support as a well-defined model system to study the bond energies between metal clusters and the oxide support as a function of particle size. The adsorption energies and growth morphologies of Cu on CeO_{2-x}(111) (where x= 0.05, 0.1 or 0.2) at 100 and 300 K were investigated using single crystal adsorption microcalorimetry together with x-ray photoelectron spectroscopy (XPS), ion scattering spectroscopy (ISS), Auger electron spectroscopy (AES), low energy electron diffraction (LEED), and sticking probability measurements. Ceria thin films (~4nm) were grown on Pt(111) single crystal. The initial heat of Cu adsorption decreased with the extent of reduction of the ceria surface. The measured heat of adsorption increases with additional Cu deposition until it reaches the Cu bulk heat of sublimation (ΔH_{sub} = 337 kJ/mol) at > 4 monolayers coverage. Interestingly, the Cu coverage required to reach ΔH_{sub} decreases as the ceria surface is reduced. These results indicate that Cu adsorbs more strongly to ceria terraces than to oxygen vacancy sites, since the primary defect for reduced ceria surfaces is oxygen vacancies, but weakens the Cu-Cu bond for particles nucleated at terraces. The growth modes of Cu on CeO_{2-x}(111) was also studied by XPS, ISS and AES. It was found that Cu grows as three dimensional particles on ceria. At 100 K the Cu particle density increased compared to 300K with a similar initial heat of adsorption, but took longer to reach the Cu heat of sublimation. The sticking probability was near unity for Cu adsorption on all these surfaces.

4:40pm **AS+BI+EM+NL+NS+SS-ThA9 Complimentary XPS and AES Analysis of MoS_3 Solid Lubricant Coatings**, ***J.R. Lince***, The Aerospace Corporation, *S.S. Alnabulsi, D.F. Paul, J.F. Moulder, J.S. Hammond*, Physical Electronics Inc.

Molybdenum disulfide (MoS_2) nanoparticles are an ideal additive in solid coating for lubricating mechanisms in vacuum environments, with widespread application in the spacecraft industry. The formation of these nanoparticles can be complex, and the use of MoS_3 nanoparticles, which are produced using a simple wet chemical synthesis is being explored as an alternate approach.[1] The use of MoS_3 as a tribological material has not been explored beyond its use as an oil additive.[2] There is new interest in investigating its potential for use in solid lubricant coatings.

To aid in the evaluation of the tribological performance of a MoS_3-formulated coating compared to MoS_2 based coatings, X-ray photoelectron spectroscopy (XPS) and Auger electron spectroscopy (AES) are utilized as complimentary techniques for the surface characterization of the contact wear regions created on the coating surface.

The unique scanning micro-focused monochromatic x-ray source was used to provide x-ray excited secondary electron images that help reveal

topographical and surface chemical information which aid in resolving and pinpointing the analysis area of interest within the contact region of the wear track that is 50 µm to 100 µm wide. The micro-XPS results provided quantitative chemical characterization that complement high spatial resolution imaging AES analysis of the sub 100 nm molybdenum sulfide particles.

Tribometer testing showed the MoS_3-formulated coating perform similar to the MoS_2-based coatings, with similar coefficients of friction and endurances in dry nitrogen. MoS_3 nanoparticles produced using simple wet chemical synthesis, and the tribology of resin-bonded MoS_3 nanoparticle coating is comparable to similarly prepared bonded coatings containing MoS_2. The surface analysis results show a lubricating effectiveness that is consistent with the production of a thin film of MoS_2 in the contact region, with an increase in the presence of sulfide relative to polysulfide in the wear track and surface segregation of lubricating species.

We will present results of micro-area XPS and AES surface analyses on worn coatings to reveal changes in composition and chemical state of the coating surface, which might explain the observed friction results of the mechanical testing.

References

P. Afanasiev, "Synthetic approaches to the molybdenum sulfide materials," Comptes Rendus Chimie, 11(1–2) (2008) 159–182.

O.P. Parenago, V.N. Bakunin, G.N. Kuz'mina, A.Yu. Suslov, and L.M. Vedeneeva, "Molybdenum Sulfide Nanoparticles as New-Type Additives to Hydrocarbon Lubricants," Doklady Chemistry, 383(1-3) (2002) 86-88.

Nanoparticle-Liquid Interfaces Focus Topic
Room: 201 B - Session NL+AS+BI-ThA

Nanoparticles with Proteins and Cells: Modelling and Measurement
Moderator: D.G. Castner, University of Washington

2:00pm **NL+AS+BI-ThA1 Nanoscale Interface Between Engineered Matter and Living Organisms: Understanding the Biological Identity of Nanosized Materials**, ***K. Dawson***, University College, Dublin **INVITED**

Nanoscale materials can interact with living organisms in a qualitatively different manner than small molecules. Crucially, biological phenomena such as immune clearance, cellular uptake and biological barrier crossing are all determined by processes on the nanometer scale. Harnessing these endogeneous biological processes (for example in creation of new nanomedicines or nanodiagnostics) will therefore require us to work on the nanoscale. This ensures that nanoscience, biology and medicine will be intimately connected for generations to come, and may well provide the best hope of tacking currently intractable diseases. These same scientific observations lead to widespread concern about the potential safety of nanomaterials in general. Early unfocussed concerns have diminished, leaving a more disciplined and balanced scientific dialogue. In particular a growing interest in understanding the fundamental principles of bionanointeractions may offer insight into potential hazard, as well as the basis for therapeutic use. Whilst nanoparticle size is important, the detailed nature of the nanoparticle interface is key to understanding interactions with living organisms. This interface may be quite complex, involving also adsorbed proteins from the biological fluid (blood, or other), leading to a 'protein corona' on the nanoparticle surface that determines its "biological identity."We discuss how this corona is formed, how it is a determining feature in biological interactions, and indeed how in many cases can undermine efforts at targeting nanoparticles using simple grafting strategies. Thus, nanoparticle interactions with living organisms cannot be fully understood without explicitly accounting for the interactions with its surroundings, i.e. the nature of the corona.

·Monopoli,M. P.; Aberg,C.;Salvati, A.;Dawson,K. A. Biomolecular Coronas Provide the Biological Identity of Nanosized Materials. *Nature Nanotechnology* **2012**,*7*,779-786.

Kim, J. A.;Aberg,C.; Salvati,A.;Dawson,K.A. Role of Cell Cycle on the Cellular Uptake and Dilution of Nanoparticles in a Cell Population. *Nature Nanotechnology* **2012**,*7*,62-68.

·Monopoli, M. P.; Walczyk, D.;Campbell,A.; Elia,G.; Lynch, I.;Baldelli Bombelli, F.;Dawson,K. A. Physical-Chemical Aspects of Protein Corona: Relevance to in Vitro and in Vivo Biological Impacts of Nanoparticles. *Journal of the American Chemical Society* **2011**,*133*,2525-2534.

·Cedervall, T.; Lynch, I.;Lindman,S.; Berggard,T.; Thulin, E.;Nilsson,H.;Dawson,K. A.;Linse,S. Understanding the Nanoparticle-Protein Corona Using Methods to Quantify Exchange Rates and Affinities of Proteins for Nanoparticles. *Proceedings of the National Academy of Sciences* **2007**,*104*,2050-2055.

2:40pm **NL+AS+BI-ThA3** ***In Silico*** **Modelling and Prediction of the Biological Effects of Nanoparticles**, ***D.A. Winkler***, *V.C. Epa, F.R. Burden,* CSIRO Materials Science & Engineering, Australia, *C. Tassa, R. Weissleder,* Harvard Medical Center, *S. Shaw*, Massachusetts General Hospital and Harvard Medical School **INVITED**

Products are increasingly incorporating nanomaterials because of their superior properties. It is estimated that 50,000 products will contain nanomaterials by 2015. However, we have a poor understanding of their potential adverse effects on workers, the public, and the environment. To assess risk, regulatory authorities need more experimental testing of nanoparticles. Computational models play a complementary role to experiments in allowing rapid prediction of potential toxicities of new and modified nanomaterials. We have generated quantitative, predictive models of cellular uptake and apoptosis induced by surface modified metal iron oxide nanoparticles for several cell types using sparse feature selection and optimal machine learning methods. We illustrate the potential of computational methods to make a contribution to nanosafety.

3:40pm **NL+AS+BI-ThA6 Quantitative Characterization of Bacterial Cell Loading with Nanoparticles**, *C. Sousa, D. Sequeira, P.M. Martins,* University of Minho, Portugal, *Y.V. Kolen'ko,* International Iberian Nanotechnology Laboratory, Portugal, *S. Lanceros-Méndez,* University of Minho, Portugal, ***D.Y. Petrovykh***, International Iberian Nanotechnology Laboratory, Portugal

The primary analytical challenge in characterizing bacterial cells loaded with nanoparticles (NPs) is that the various methods that are traditionally used to measure cells or NPs separately are not readily applied to the mixed samples. These complex samples may contain, for example, a mixture of free NPs, NP-loaded cells, and cells without NPs, while the relative concentrations of NPs and cells or the average number of NPs loaded in one cell is not always known or readily established. Accordingly, methods for separating the different sample components have to be developed and validated before the component of interest (NP-loaded cells in most cases) can be characterized. The final challenge is determining the localization of NPs in and around the cells, as for some applications in sensing and nanomedicine NPs bound to cells externally can be the goal, whereas for exploiting physical properties of NPs, e.g., to induce hyperthermia, maximizing the internalization of NPs by cells can be advantageous.

Our approach to investigating these complex analytical challenges is based on using model systems that are amendable to quantitative characterization by complementary methods, both separately and when mixed as indicated above. Specifically, we are using *Staphyloccus aureus* as model bacterial cells, in part because the typical 500 nm diameter of *S. aureus* cells is within the size range of NP aggregates or large NPs, therefore, the same microscopy and spectroscopy methods can be applied to both components of mixed cell-NP samples. We use gold NPs as the primary model NPs because the strong plasmon peak enables their characterization in solution, while the high density and atomic number of gold can be helpful during separation and for characterization by electron microscopy and spectroscopy. Superparamagnetic iron-oxide NPs with different organic shells are used as a second type of model NPs. We will describe the use of multiple complementary microscopy and spectroscopy techniques for developing, validating, and quantifying protocols for cell-NP separation and for characterization of cell loading by NPs.

4:00pm **NL+AS+BI-ThA7 Protein-Corona: A New Gateway to Disease Therapeutics**, ***K. Giri***, *P. Mukherjee, M. Zimmermann, S. Khader, B. Madden, D. McCormick*, Mayo Clinic

Nanomedicine is a burgeoning field with immense potential in disease therapeutics, diagnosis and imaging. However, an inevitable phenomenon regarding the use of nanoparticles (NPs) *in vivo* is the adsorption of proteins to its surface to form a layer called the "protein corona". The concept of synthetic vs. biological identity of the NPs has emerged. Studies have reported that the acquired biological identity of NPs due to its protein corona influences not just the interaction of the NPs with its targets but also its fate. Among all the NPs that are currently being investigated in nanomedicine, gold nanoparticles (GNPs) are unique in that they posses strong affinity to bind to SH and NH2 containing molecules. Therefore, proteins by virtue of having cysteine and lysine residues function as unique substrates to bind to GNPs. We hypothesize that the proteome and secretome of cancer cells may include low abundance proteins that escape detection by conventional methods. Enrichment and identification of these proteins may play a critical role in understanding the pathophysiology of disease development and open new avenues for treatment. Our aim was to study the formation of protein corona on GNP surface as a unique way to enrich and identify low abundance proteins that can serve as new

therapeutic targets for ovarian cancer. Understanding the interaction of proteins on GNP surface is important as it will guide modulation of protein corona formation for protein enrichment based on physicochemical properties and structure. Here, we present a systematic study of protein corona using 20 nm GNPs. We studied the binding of proteins from lysates derived from two ovarian cell lines, namely OSE (non-cancerous) and A2780 (cancerous). We followed the evolution of the corona for 24 hrs to account for the dynamic and competitive binding of proteins on the NP surface. We characterized the corona at 5 mins, 15 mins, 1hr, 6hrs and 24 hrs using UV-vis spectroscopy, dynamic light scattering, electron microscopy and ζ-potential measurements and identified corona constituents by mass spectroscopy. We focused on understanding what drives protein adsorption to the NP surface. Lastly, we identified low abundance proteins from the A2780 cell line that were enriched on GNP surface as a proof of concept study to demonstrate that protein corona can be effectively utilized for study of disease and its therapeutics.

Nanoparticle-Liquid Interfaces Focus Topic
Room: 201 B - Session NL+AS+BI+SA-FrM

Emerging Methods to Identify and Measure Nanomaterials in Biological Environments

Moderator: G. Ceccone, European Commission, Joint Research Centre, IHCP, Italy

8:20am **NL+AS+BI+SA-FrM1 3D Views of Hydrated Biological Cells with Soft X-ray Tomography**, ***C.A. Larabell***, University of California, San Francisco **INVITED**

SXT is similar in concept to the well-established medical diagnostic technique, computed axial tomography (CAT), except SXT is capable of imaging with a spatial resolution of 50 nm, or better. We examine whole, hydrated cells (between 10-15 μm thick), eliminating the need for time-consuming and potentially artifact-inducing embedding and sectioning procedures. Cells are rapidly frozen then imaged using photons with energies between the K shell absorption edges of carbon (284 eV, λ=4.4 nm) and oxygen (543 eV, λ=2.3 nm). In this energy range, photons readily penetrate the aqueous environment while encountering significant absorption from carbon- and nitrogen-containing organic material. Consequently organic material absorbs approximately an order of magnitude more strongly than water, producing a quantifiable natural contrast image of cellular structures. By collecting images from multiple angles through 360 degrees of rotation, SXT reconstructions yield information at isotropic resolution.

Images are formed using unique optics called zone plates (ZP). An X-ray ZP optic consists of a number of concentric nanostructured metal rings, or zones, formed on a thin X-ray transmissive silicon nitride membrane. The width of the outermost ring determines the spatial resolution of the ZP lens, whereas the thickness of the rings determines the focusing efficiency. In our microscope, we use a condenser ZP lens with an overall diameter of 1 cm and an outer zone width of 50 nm. The high-resolution objective ZP lens has a diameter of 63 μm, 618 zones, a focal length of 650 μm at 2.4 nm wavelength, and an outer zone width of 50 nm.

Because SXT is fast (~ 5 min per tomographic data set), we can examine large numbers of cells. Since organic material absorbs approximately an order of magnitude more strongly than water, a unique and quantifiable natural contrast image of cellular structures is generated. X-ray absorption follows Beer's Law, therefore the absorption of photons is linear and a function of the biochemical composition at each point in the cell. As a result, a linear absorption coefficient (LAC) value of each voxel can be calculated. For example, lipid drops with high concentrations of carbon are more highly absorbing (LAC=0.7 mm^{-1}) than fluid-filled vesicles (LAC=0.2 mm^{-1}). We can determine the position of specific molecules by overlaying fluorescence microscopy signals on cell structures obtained with x-ray imaging. In addition, we can directly determine the locations and numbers of metal probes throughout the cell.

9:20am **NL+AS+BI+SA-FrM4 Ultrathin Electron Transparent Membranes as a Platform for Scanning Electron and Photoelectron Imaging and Spectroscopy of Fully Hydrated Nanoparticles**, ***X.M. Ma***, *J. Geisler-Lee*, Southern Illinois University Carbondale, *M. Amati, L. Gregoratti*, Sincrotrone Trieste, Italy, *S. Guenther*, Technical University Muenchen, Germany, *M. Kiskinova*, Sincrotrone Trieste, Italy, *A. Kolmakov*, Southern Illinois University Carbondale

The increased use of engineered nanoparticles (ENPs) in biomedical applications and their inevitable release into the environment has prompted considerable need to study of their uptake, accumulation and transport inside biological tissue and in plants. This is particularly true for addressing the ENPs fate on a cellular level which inevitably requires the microscopy approach. For long time optical microscopy with the resolution in the order of 100 nm was the major tool available. Better resolution can be readily achieved with traditional transmission (TEM) or scanning (SEM) electron microscopy. However, it requires histological sample treatments such as fixation, staining, dehydration, freezing etc which excludes *in vivo (in situ)* modes of observations and can alter their native morphology, functionality and living cycles. Different from standard environmental SEM, where the near sample pressure is limited by ca few tens of Torr, we are actively working on fabrication and tests of electron transparent membranes for ambient pressure electron spectromicroscopy and its application to fully hydrated samples for phytotoxicity, and materials research. Such enclosed environmental cells, equipped with 50-100 nm windows transparent for 10-20 keV electrons, can maintain the sample at atmospheric pressure and/or fully hydrated. This approach is beneficial compared with dry methods since *in vivo* SEM/TEM observations at nanoscale can be performed. Using this methodology, we were able to image the uptake of silver (Ag) NPs by living Arabidopsis roots on a cellular level. It was shown that NPs with the sizes larger than 20 nm accumulate preferably on the surface of the cellular walls and do not to traverse the plant cell membrane.

Recent developments in high yield fabrication and handling protocols of ultrathin (~1 nm) membranes, such as graphene or graphene oxide sheets with thicknesses comparable to the effective attenuation length (EAL) of 200-1000 eV electrons opened the opportunity to perform traditional XPS (X-ray Photoelectron Spectroscopy) and AES (Auger Electron Spectroscopy) at the interfaces between the membrane and fully hydrated samples. Using model water solutions and NPs, we report here on major design principles of such cells as well on first spectral demonstrations, advantages and limitations of this new technique.

9:40am **NL+AS+BI+SA-FrM5 Small-angle X-ray Scattering Investigation of Functional Materials at Inorganic-Marcromolecular Interfaces**, ***T.W. van Buuren***, *T.M. Willey, J.R.I. Lee, I.C. Tran, M. Bagge-Hansen*, Lawrence Livermore National Laboratory

Development in nanoscale engineering has enabled bioelectronics that can mimic and/or interact with the biological systems. Lipid bilayer-functionalized Si nanowires are considered as a promising candidate for the construction of bioelectrochemical devices. These biomimetic lipid bilayers serve as a general host matrix for bio-functional components such as membrane proteins. Though meaningful technological advancement of these materials has been made, critical questions about their structural and chemical composition remain. Small angle x-ray scattering (SAXS) experiments are used to investigate the structure of the lipid bilayers on Si nanowires, which provide information on the overall 1-D bilayer structure, the effect of substrate curvature on the lipid packing and local self-organization. The SAXS derived lateral-averaged characterizations are then corroborated with local arrangements of lipid bilayers on Si nanowires revealed by Scanning Transmission X-ray Spectroscopy (STXM). The results provide insights into a number of unresolved questions that are crucial for the comprehensive understanding this class of materials.

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Authors Index

Bold page numbers indicate the presenter

— A —

Aldinger, J.: AS+BI+EM+NL+NS+SS-ThA1, 14
Alexander, C.: BI+AS+IS+NL-MoM2, 2
Alexander, M.R.: BI+AS+IS+NL-MoM10, 3; BI+AS+IS+NL-MoM2, 2; BI+AS+IS+NL-MoM8, 3
Alnabulsi, S.S.: AS+BI+EM+NL+NS+SS-ThA9, 14
Amagai, M.: BI+AI+AS+BA+IA+NL+NS+SP-WeA1, 8
Amati, M.: NL+AS+BI+SA-FrM4, 17
Anderson, D.G.: BI+AS+IS+NL-MoM10, 3; BI+AS+IS+NL-MoM8, 3
Antosiewicz, T.: BI+NL+NS+SS-ThM6, 12

— B —

Backus, E.H.G.: IA+AI+BI+IS+NL+SS-MoA3, 4
Bagge-Hansen, M.: NL+AS+BI+SA-FrM5, 17
Baimpos, T.: IA+AI+BI+IS+NL+SS-MoA11, **4**
Baio, J.E.: BA+AI+AS+BI+IS+NL-MoM5, **1**
Bakker, H.J.: IA+AI+BI+IS+NL+SS-MoA3, 4
Banerjee, S.: AS+BI+EM+NL+NS+SS-ThA1, **14**
Baran, N.: BI+AS+IS+NL-MoM3, 2
Behafarid, F.: AS+BI+EM+NL+NS+SS-ThA3, 14
Belchik, S.: BI+AI+AS+BA+IA+NL+NS+SP-WeA2, 8
Belfort, G.: BI+AS+BA+NL-TuM10, 6
Bell, N.C.: AS+BI+EM+NL+NS+SS-ThM5, 10
Bischoff, F.R.: BI+NL+NS+SS-ThM11, 13
Bluhm, H.: IA+AI+BI+IS+NL+SS-MoA10, 4
Bonn, M.: BA+AI+AS+BI+IS+NL-MoM10, 1; BA+AI+AS+BI+IS+NL-MoM5, 1; IA+AI+BI+IS+NL+SS-MoA3, **4**
Bray, K.R.: AS+BI+EM+NL+NS+SS-ThM12, **11**
Breitling, F.: BI+NL+NS+SS-ThM11, 13
Burden, F.R.: NL+AS+BI-ThA3, 15
Buttery, L.: BI+AS+IS+NL-MoM2, 2

— C —

Cai, X.: BA+AI+AS+BI+IS+NL-MoM8, 1
Calzolai, L.: AS+BI+EM+NL+NS+SS-ThM10, 11
Campbell, C.T.: AS+BI+EM+NL+NS+SS-ThA6, 14
Canales, M.: BI+AI+AS+BA+IA+NL+NS+SP-WeA12, **9**
Castner, D.G.: AS+BI+EM+NL+NS+SS-ThM6, **10**; BI+AI+AS+BA+IA+NL+NS+SP-WeA8, 8
Ceccone, G.: AS+BI+EM+NL+NS+SS-ThM10, **11**
Chaabane, N.: AS+BI+EM+NL+NS+SS-ThA4, 14
Chait, B.T.: BI+AS+BA+NL-TuM10, 6
Chang, C.: BI+AS+IS+NL-MoM8, 3
Chen, S.: AS+BI+EM+NL+NS+SS-ThM11, 11; BI+NL+NS+SS-ThM6, **12**
Chilkoti, A.: BI+NL+NS+SS-ThM9, **12**
Christophis, C.: BI+AS+IS+NL-MoM3, 2
Cohen, S.R.: BI+AI+AS+BA+IA+NL+NS+SP-WeA9, **9**
Connell, S.D.: BI+AI+AS+BA+IA+NL+NS+SP-WeA10, 9
Corkery, R.: IA+AI+BI+IS+NL+SS-MoA7, 4

— D —

Dahint, R.: BI+NL+NS+SS-ThM11, **13**
Davies, M.C.: BI+AS+IS+NL-MoM10, 3; BI+AS+IS+NL-MoM8, 3
Dawson, K.: NL+AS+BI-ThA1, **15**
De Battice, L.: BI+AS+BA+NL-TuM5, 6
DeCerbo, J.N.: AS+BI+EM+NL+NS+SS-ThM12, 11
Denning, C.: BI+AS+IS+NL-MoM10, 3
Dennis, R.: AS+BI+EM+NL+NS+SS-ThA1, 14
Dixon, M.P.: BI+AI+AS+BA+IA+NL+NS+SP-WeA11, 9
Donald, D.R.: AS+BI+EM+NL+NS+SS-ThM11, **11**
Doughty, B.: BA+AI+AS+BI+IS+NL-MoM1, 1
Dufresne, C.: BI+AS+IS+NL-MoM4, **2**

— E —

Eisenthal, K.B.: BA+AI+AS+BI+IS+NL-MoM1, **1**
Engelhard, M.: AS+BI+EM+NL+NS+SS-ThM11, 11
Enochson, L.: BI+AS+BA+NL-TuM6, 6
Epa, V.C.: BI+AS+IS+NL-MoM8, **3**; NL+AS+BI-ThA3, 15
Evans, S.D.: BI+AI+AS+BA+IA+NL+NS+SP-WeA10, 9

— F —

Faingold, A.: BI+AI+AS+BA+IA+NL+NS+SP-WeA9, 9
Faubel, M.: IA+AI+BI+IS+NL+SS-MoA10, 4
Frank, C.W.: BI+AS+BA+NL-TuM11, **7**
Fröhlich, J.: BA+AI+AS+BI+IS+NL-MoM10, 1
Frost, R.: BI+AS+BA+NL-TuM5, 6

— G —

Gamble, L.J.: BI+AI+AS+BA+IA+NL+NS+SP-WeA7, **8**; BI+AI+AS+BA+IA+NL+NS+SP-WeA8, 8
Geiger, F.: BA+AI+AS+BI+IS+NL-MoM3, **1**
Geisler-Lee, J.: NL+AS+BI+SA-FrM4, 17
Gellman, A.J.: AS+BI+EM+NL+NS+SS-ThM9, 11
Gettel, D.: BI+NL+NS+SS-ThM3, 12
Gibbs-Davis, J.M.: IA+AI+BI+IS+NL+SS-MoA6, **4**
Gilliland, D.: AS+BI+EM+NL+NS+SS-ThM10, 11
Giri, K.: NL+AS+BI-ThA7, **15**
Gold, J.: BI+AS+BA+NL-TuM6, 6
Graham, D.J.: BI+AI+AS+BA+IA+NL+NS+SP-WeA8, **8**
Grainger, D.W.: AS+BI+EM+NL+NS+SS-ThM1, **10**
Gregoratti, L.: NL+AS+BI+SA-FrM4, 17
Guenther, S.: NL+AS+BI+SA-FrM4, 17
Gupta, S.: BI+AS+IS+NL-MoM11, 3
Guvenc, H.O.: BI+NL+NS+SS-ThM11, 13

— H —

Hammond, J.S.: AS+BI+EM+NL+NS+SS-ThA9, 14; BI+AI+AS+BA+IA+NL+NS+SP-WeA1, **8**
Hanke, M.: BI+AS+IS+NL-MoM3, 2
Hayama, R.: BI+AS+BA+NL-TuM10, 6
Heath, G.R.: BI+AI+AS+BA+IA+NL+NS+SP-WeA10, **9**
Heller, G.: BI+AS+BA+NL-TuM4, **6**
Hemminger, J.C.: IA+AI+BI+IS+NL+SS-MoA10, 4
Hemmingson, S.L.: AS+BI+EM+NL+NS+SS-ThA6, 14
Henderson, S.: AS+BI+EM+NL+NS+SS-ThA1, 14
Hill, E.: BI+AI+AS+BA+IA+NL+NS+SP-WeA2, 8
Ho, A.D.: BI+AS+IS+NL-MoM3, 2
Hockenbery, D.: BI+AI+AS+BA+IA+NL+NS+SP-WeA7, 8
Hook, A.L.: BI+AS+IS+NL-MoM8, 3

— I —

Ishizaki, I.: BI+AI+AS+BA+IA+NL+NS+SP-WeA1, 8

— J —

James, T.E.: AS+BI+EM+NL+NS+SS-ThA6, **14**
Jiao, C.Q.: AS+BI+EM+NL+NS+SS-ThM12, 11
Johal, M.S.: BI+AI+AS+BA+IA+NL+NS+SP-WeA11, 9; BI+AS+BA+NL-TuM4, 6
Johnson, L.E.: BI+AI+AS+BA+IA+NL+NS+SP-WeA11, 9
Jupille, J.: AS+BI+EM+NL+NS+SS-ThA4, **14**

— K —

Käll, M.: BI+NL+NS+SS-ThM6, 12
Kasemo, B.H.: BI+AS+BA+NL-TuM1, **6**
Kawasaki, H.: BI+AI+AS+BA+IA+NL+NS+SP-WeA1, 8
Kazer, S.M.: BA+AI+AS+BI+IS+NL-MoM1, 1
Khader, S.: NL+AS+BI-ThA7, 15
Khosla, N.: AS+BI+EM+NL+NS+SS-ThM9, 11
King, A.J.: BI+AI+AS+BA+IA+NL+NS+SP-WeA12, 9
Kingshott, P.: BI+AS+IS+NL-MoM1, 2
Kiskinova, M.: NL+AS+BI+SA-FrM4, 17
Kolen'ko, Y.V.: NL+AS+BI-ThA6, 15
Kolmakov, A.: NL+AS+BI+SA-FrM4, 17
Kraft, M.L.: BI+AI+AS+BA+IA+NL+NS+SP-WeA3, **8**
Kraut, R.: BI+NL+NS+SS-ThM3, 12
Kubo, A.: BI+AI+AS+BA+IA+NL+NS+SP-WeA1, 8
Kunze, A.: BI+AS+BA+NL-TuM6, 6
Kwok, S.J.J.: BA+AI+AS+BI+IS+NL-MoM1, 1

— L —

La Spina, R.: AS+BI+EM+NL+NS+SS-ThM10, 11
Lanceros-Méndez, S.: NL+AS+BI-ThA6, 15
Lang, X.: BA+AI+AS+BI+IS+NL-MoM6, **1**
Langer, R.: BI+AS+IS+NL-MoM10, 3; BI+AS+IS+NL-MoM8, 3
Larabell, C.A.: NL+AS+BI+SA-FrM1, **17**
Lazzari, R.: AS+BI+EM+NL+NS+SS-ThA4, 14
Lee, J.R.I.: NL+AS+BI+SA-FrM5, 17
Lee, V.: AS+BI+EM+NL+NS+SS-ThA1, 14
Liang, C.-K.: AS+BI+EM+NL+NS+SS-ThM3, 10
Liedberg, B.: BI+NL+NS+SS-ThM3, 12
Lince, J.R.: AS+BI+EM+NL+NS+SS-ThA9, **14**
Lindahl, A.: BI+AS+BA+NL-TuM6, 6
Liu, B.: BI+AI+AS+BA+IA+NL+NS+SP-WeA2, 8
Lyubovitsky, J.G.: BA+AI+AS+BI+IS+NL-MoM6, 1

— M —

Ma, X.M.: NL+AS+BI+SA-FrM4, **17**
Madden, B.: NL+AS+BI-ThA7, 15
Mantovani, G.: BI+AS+IS+NL-MoM2, 2
Margarella, A.M.: IA+AI+BI+IS+NL+SS-MoA10, 4
Marshall, M.: BI+AI+AS+BA+IA+NL+NS+SP-WeA2, 8
Martins, P.M.: NL+AS+BI-ThA6, 15
McCormick, D.: NL+AS+BI-ThA7, 15
Meyerbröker, N.: BI+NL+NS+SS-ThM5, **12**
Minelli, C.: AS+BI+EM+NL+NS+SS-ThM5, **10**
Mistry, H.: AS+BI+EM+NL+NS+SS-ThA3, **14**
Morrish, F.: BI+AI+AS+BA+IA+NL+NS+SP-WeA7, 8
Moulder, J.F.: AS+BI+EM+NL+NS+SS-ThA9, 14
Mukherjee, P.: NL+AS+BI-ThA7, 15
Mulvaney, S.P.: BI+NL+NS+SS-ThM12, 13
Munusamy, P.: AS+BI+EM+NL+NS+SS-ThM11, 11

— N —

Nagao, K.: BI+AI+AS+BA+IA+NL+NS+SP-WeA1, 8
Nesterov-Mueller, A.: BI+NL+NS+SS-ThM11, 13
Netz, R.: IA+AI+BI+IS+NL+SS-MoA8, **4**
Nilebäck, E.: BI+AS+BA+NL-TuM6, **6**

— O —

Oglecka, K.: BI+NL+NS+SS-ThM3, 12
Ohashi, Y.: BI+AI+AS+BA+IA+NL+NS+SP-WeA1, 8
Ondeck, N.: AS+BI+EM+NL+NS+SS-ThM9, 11

— P —

Pandey, R.: BA+AI+AS+BI+IS+NL-MoM10, 1
Parikh, A.N.: BI+NL+NS+SS-ThM3, **12**
Patel, A.K.: BI+AS+IS+NL-MoM10, **3**
Paul, D.F.: AS+BI+EM+NL+NS+SS-ThA9, 14

Perrine, K.A.: IA+AI+BI+IS+NL+SS-MoA10, **4**
Persson, M.: BI+AS+BA+NL-TuM5, 6
Petrovykh, D.Y.: NL+AS+BI-ThA6, **15**
Piatkowski, L.: IA+AI+BI+IS+NL+SS-MoA3, 4
Porter, A.: AS+BI+EM+NL+NS+SS-ThM11, 11
Pöschl, U.: BA+AI+AS+BI+IS+NL-MoM10, 1
Prusnick, T.: BI+AI+AS+BA+IA+NL+NS+SP-WeA12, 9

— R —

Radunskaya, A.: BI+AS+BA+NL-TuM4, 6
Rafati, A.: AS+BI+EM+NL+NS+SS-ThM6, 10
Rao, Y.: BA+AI+AS+BI+IS+NL-MoM1, 1
Reed, S.M.: BI+NL+NS+SS-ThM2, **12**
Renaud, G.: AS+BI+EM+NL+NS+SS-ThA4, 14
Reviakine, I.: BI+AS+IS+NL-MoM11, **3**
Robinson, J.T.: BI+NL+NS+SS-ThM12, 13
Robinson, M.: BI+AI+AS+BA+IA+NL+NS+SP-WeA7, 8
Roldan Cuenya, B.: AS+BI+EM+NL+NS+SS-ThA3, 14
Rosenhahn, A.: BI+AS+IS+NL-MoM3, **2**
Rossi, F.: AS+BI+EM+NL+NS+SS-ThM10, 11
Rout, M.P.: BI+AS+BA+NL-TuM10, 6

— S —

Sanborn, J.: BI+NL+NS+SS-ThM3, 12
Sazinsky, M.: BI+AS+BA+NL-TuM4, 6
Schirwitz, C.: BI+NL+NS+SS-ThM11, 13
Schultz, B.J.: AS+BI+EM+NL+NS+SS-ThA1, 14
Schweikert, E.A.: AS+BI+EM+NL+NS+SS-ThM3, **10**
Sequeira, D.: NL+AS+BI-ThA6, 15
Shahar, R.: BI+AI+AS+BA+IA+NL+NS+SP-WeA9, 9
Shard, A.G.: AS+BI+EM+NL+NS+SS-ThM5, 10
Shaw, S.: NL+AS+BI-ThA3, 15
Sheehan, P.E.: BI+NL+NS+SS-ThM12, **13**
Shen, Y.R.: IA+AI+BI+IS+NL+SS-MoA1, **4**
Shukla, N.: AS+BI+EM+NL+NS+SS-ThM9, **11**
Simoes, F.A.: BI+AS+IS+NL-MoM2, **2**
Soares, EA.: AS+BI+EM+NL+NS+SS-ThA4, 14
Somorjai, G.A.: BA+AI+AS+BI+IS+NL-MoM8, **1**
Sorci, M.: BI+AS+BA+NL-TuM10, **6**
Sousa, C.: NL+AS+BI-ThA6, 15
Spampinato, V.: AS+BI+EM+NL+NS+SS-ThM10, 11
Stadler, V.: BI+NL+NS+SS-ThM11, 13
Stine, R.: BI+NL+NS+SS-ThM12, 13
Sturve, J.: BI+AS+BA+NL-TuM5, 6
Sundblom, A.: BI+AS+BA+NL-TuM5, 6
Svedendhal, M.: BI+NL+NS+SS-ThM6, 12
Svedhem, S.: BI+AS+BA+NL-TuM5, **6**; BI+AS+BA+NL-TuM6, 6
Szakal, C.: AS+BI+EM+NL+NS+SS-ThM4, **10**

— T —

Tamanaha, C.R.: BI+NL+NS+SS-ThM12, 13
Tassa, C.: NL+AS+BI-ThA3, 15
Taubert, I.: BI+AS+IS+NL-MoM3, 2
Tran, I.C.: NL+AS+BI+SA-FrM5, 17
Turro, N.T.: BA+AI+AS+BI+IS+NL-MoM1, 1
Tymchenko, N.: BI+AS+BA+NL-TuM6, 6
Tyrode, E.C.: IA+AI+BI+IS+NL+SS-MoA7, **4**

— V —

Valtiner, M.: IA+AI+BI+IS+NL+SS-MoA11, 4
van Buuren, T.W.: NL+AS+BI+SA-FrM5, **17**
Van Spyk, M.H.C.: IA+AI+BI+IS+NL+SS-MoA10, 4
Verkhoturov, S.V.: AS+BI+EM+NL+NS+SS-ThM3, 10

— W —

Wagner, H.D.: BI+AI+AS+BA+IA+NL+NS+SP-WeA9, 9
Wagner, J.: BI+NL+NS+SS-ThM11, 13
Wallin, M.: BI+AS+BA+NL-TuM5, 6
Wallin, P.: BI+AS+BA+NL-TuM6, 6
Wang, C.W.: AS+BI+EM+NL+NS+SS-ThM11, 11
Wang, P.Y.: BI+AS+IS+NL-MoM1, **2**
Wang, Y.-C.: AS+BI+EM+NL+NS+SS-ThM6, 10
Wayment-Steele, H.K.: BI+AI+AS+BA+IA+NL+NS+SP-WeA11, **9**
Weidner, T.: BA+AI+AS+BI+IS+NL-MoM10, **1**; BA+AI+AS+BI+IS+NL-MoM5, 1
Weiner, S.: BI+AI+AS+BA+IA+NL+NS+SP-WeA9, 9
Weissleder, R.: NL+AS+BI-ThA3, 15
Weitz, D.A.: BI+AS+IS+NL-MoM5, **2**
Willey, T.M.: NL+AS+BI+SA-FrM5, 17
Williams, P.: BI+AS+IS+NL-MoM8, 3
Winkler, D.A.: BI+AS+IS+NL-MoM8, 3; NL+AS+BI-ThA3, **15**
Winter, B.: IA+AI+BI+IS+NL+SS-MoA10, 4
Wiseman, M.E.: BI+AS+BA+NL-TuM11, 7
Wuchter, P.: BI+AS+IS+NL-MoM3, 2

— Y —

Yang, J.: BI+AS+IS+NL-MoM8, 3
Yang, L.: BI+AI+AS+BA+IA+NL+NS+SP-WeA2, 8
Yen, L.B.: AS+BI+EM+NL+NS+SS-ThM11, 11
Yousaf, M.N.: BI+AS+IS+NL-MoM9, **3**
Yu, X.Y.: BI+AI+AS+BA+IA+NL+NS+SP-WeA2, **8**

— Z —

Zhang, Z.: IA+AI+BI+IS+NL+SS-MoA3, 4
Zharnikov, M.: BI+NL+NS+SS-ThM5, 12
Zhu, Z.: BI+AI+AS+BA+IA+NL+NS+SP-WeA2, 8
Zimmermann, M.: NL+AS+BI-ThA7, 15
Zwang, T.: BI+AS+BA+NL-TuM4, 6