Biomaterial Interfaces
Room: Milo - Session BI-TuM

Biomaterial & Wet Interface Characterization
Moderator: Xiaoying Yu, Pacific Northwest National Laboratory

8:00am **BI-TuM1 Engineering of Bio-Nano Interfaces with Self-Assembled Peptides**, ***Yuhei Hayamizu***, Tokyo Institute of Technology, Japan **INVITED**

Developing elegant hybrid systems of biological molecules on two-dimensional nanomaterials is a key in creating novel bio-nanoelectronic devices. Biomolecules self-assembling into ordered structures on these nanomaterials offer a novel bottom-up approach, where organized supramolecular architectures spatially govern the electronics of nanomaterials. Despite the enormous potential in bridging nano- and bio-worlds at the molecular scale, no work has yet realized a way to control electronic properties of nanomaterials by these biomolecular structures. Our research target is the control of the interface between biotechnology and nanotechnology. In this work, we employ solid binding peptides or artificially-designed peptides which have specific binding affinities to solid surfaces and an ability to form peptide nanostructures on atomically flat surfaces [1,2]. These peptides self-assemble monolayer-thick long-range ordered nanostructures on surfaces of single-layer graphene, and on other two-dimensional materials. We observed that self-assembled peptides on a single layer graphene modify its conductivity depending on their assembled structures.

[1] C. R. So, Y. Hayamizu, H. Yazici, C. Gresswell, D. Khatayevich, C. Tamerler, and M. Sarikaya, "Controlling Self Assembly of Engineered Peptides on Graphite by Rational Mutation," ACS Nano, **6** (2) 1648-1656 (2012)

[2] T. R. Page, Y. Hayamizu, C. R. So, and M. Sarikaya, "Electrical Detection of Biomolecular Adsorption on Sprayed Graphene Sheets," Biosens. Bioelectron., **33** (1) 304-308 (2012)

8:40am **BI-TuM3 Peptide Control of Biological Membranes — A Molecular View on Lipid Structure, Peptide Folding and Hydration**, ***Johannes Franz***, *D. Schach*, Max Planck Institute for Polymer Research, *J.E. Baio*, Oregon State University, *D.J. Graham*, *D.G. Castner*, University of Washington, USA, *M. Bonn*, Max Planck Institute for Polymer Research, *T. Weidner*, Max Planck Institute for Polymer Research, Germany

The cell membrane is the most important biological surface as its interaction with peptides is an integral part of transport, communication, energy transduction and survivability. However, an intrinsic difficulty in monitoring peptide interaction with membranes is the required surface sensitivity. Sum frequency generation (SFG) vibrational spectroscopy is well suited to study protein monolayers at lipid surfaces[1] because of its inherent surface specificity and is used to investigate molecular interactions of peptides with model membranes. In this study, three different peptides are shown to interact with model membranes in very different ways.

The internalization mechanism of the negatively charged cell-penetrating peptide SAP(E) is proposed as an aggregation on the cell surface followed by an endocytic uptake. Our data suggest peptide affinity is strongly dependent on the lipid headgroup charge with phosphocholine having the strongest interaction with SAP(E). Moreover, the interaction is limited to the headgroup region with no further insertion observable proving the first step of the proposed uptake mechanism. These findings were supported with complementary surface-sensitive UHV-techniques, i.e. X-ray photoelectron spectroscopy (XPS), near edge X-ray absorption fine structure (NEXAFS) spectroscopy and time-of-flight secondary ion mass spectrometry (ToF-SIMS).

In contrast, viral fusion proteins can disrupt membranes and escape from endosomes when triggered at low pH. We are interested in the interaction of GALA, a peptide mimicking viral fusion proteins. While the peptide is unfolded and inactive around neutral pH, the sequence folds into its active α-helical state at lower pH and causes membrane leakage. We follow GALA activity at the molecular level and probe peptide folding as well as the disturbance and hydration of individual leaflets within model bilayers.

Besides binding to and shredding bilayers, peptides can also help stabilize lipid membranes. For example, bovine serum albumin and specific antifreeze proteins can maintain cell membrane integrity at low temperatures. We briefly discuss preliminary results about the effects of protein-lipid interactions on the temperature stability of lipid mono- and bilayers.

[1] Chen, X., Chen, Z., BBA 1758 (2006), 1257-1273.

9:20am **BI-TuM5 Development and Characterization of Tunable Porous 3D Materials for Biomedical and Environmental Applications**, *M.J. Hawker*, *A. Pegalajar-Jurado*, *M.N. Mann*, ***Ellen Fisher***, Colorado State University

Porous 3D materials are used in a range of applications from tissue engineering to water filtration to drug delivery systems. In many instances, the surface properties of these materials are not, however, ideal for the intended applications. Low temperature plasmas offer a versatile method for delivering tailored functionality to a range of materials. Despite the vast array of choices offered by plasma processing techniques, there remain a significant number of hurdles that must be overcome to allow this methodology to realize its full potential, especially with porous 3D structures such as membranes and scaffolds. Challenges include ensuring uniform composition following treatment, controlling morphology and damage, characterization of both the external and internal features as well as accurate assessment of bioactivity. Here, we present results demonstrating the relative biocompatibility of various plasma treatment strategies for polymeric membranes and scaffolds. Results from mammalian cell (human dermal fibroblasts) cytotoxicity experiments (MTS, Live/Dead, plating efficiency and morphological studies) will be demonstrated for a range of plasma treated surfaces including bio-nonreactive (e.g. fluorocarbon coated) and bio-reactive (e.g. H_2O plasma treated) 3D poly(e-caprolactone) scaffolds. All materials were characterized using X-ray photoelectron spectroscopy, scanning electron microscopy and contact angle measurements. Additional results demonstrating the efficacy of our plasma treatments in creating low fouling antimicrobial membranes and scaffolds will also be presented. Here, tunable hydrophilic surface modification strategies for different polymeric architectures are evaluated, including plasma modification of NO-releasing materials, ultrafiltration membranes, and polylactic acid constructs. Notably, many of the strategies result in 3D constructs that enhance cell growth and proliferation, retain antibacterial properties and offer promising results for applications including tissue engineering, noble water filtration systems, and advanced biomedical devices.

9:40am **BI-TuM6 The Formation of a Self-Hydrated Artificial Phospholipid Membrane on Ultra-Thin Chitosan Layer Deposited from the Gas-Phase**, *M.J. Retamal*, *M.A. Cisternas*, Pontificia Universidad Católica de Chile, Instituto de Física, Chile, *S.E. Gutierrez-Maldonado*, *T. Perez-Acle*, Fundación Ciencia & Vida, Chile, *B. Seifert*, Pontificia Universidad Católica de Chile, Instituto de Física, Chile, *M. Busch*, *P. Huber*, Hamburg University of Technology (TUHH), Germany, *U.G. Volkmann*, Pontificia Universidad Católica de Chile, Instituto de Física, Chile, ***Valeria del Campo***, Universidad Técnica Federico Santa María, Chile

The design of interfaces between solid surfaces and biological molecules such as membranes and/or proteins using $Si(100)/SiO_2$, a.k.a. *bio-silicon interfaces*, is an important and rapid developing area of both scientific and applied research. Preparation and characterization of artificial biological membranes is a necessary step for the formation of nano-devices or sensors. A soft hydrophilic polymer cushion could help to provide a "bio-mimetic" environment for the membrane and for membrane-spanning proteins. Several candidates to be used as soft-cushion polymers are currently under research, such as dextran, hyaluronic acid and other polysaccharides. Chitosan is a linear polysaccharide obtained by the deacetylation of chitin, which can be found in the shells of crustaceans, exoskeletons of insects, fungi and plants, thus being very easy to obtain from nature at low cost. In the last years, device manufacturing for medical applications adapted the so-called bottom-up approach, from nanostructures to larger devices. We describe the formation and characterization of a phospholipid bilayer (DPPC) on a mattress of a polysaccharide (Chitosan) that keeps the membrane hydrated. The deposition of Chitosan (~25Å) and DPPC (~60Å) was performed from the gas phase in high vacuum onto a substrate of Si(100) covered with its native oxide layer. The layer thickness was controlled *in situ* using Very High Resolution Ellipsometry (VHRE). Raman spectroscopy studies show that neither Chitosan nor DPPC molecules decompose during evaporation. With VHRE and Atomic Force Microscopy (AFM) we have been able to detect phase transitions in the membrane. The presence of the Chitosan interlayer as a water reservoir is essential for both DPPC bilayer formation and stability, favoring the appearance of phase transitions. Our experiments show that the proposed sample preparation from the gas phase is reproducible and provides a natural environment for the DPPC bilayer.

We thank for financial support under FONDECYT grant No. 1100882 and 1141105, and acknowledge a CONICYT scholarship of M.J.R. (Ph.D),

M.A.C. (Master) and S.E.G.M. (Ph.D). U.G.V and T.P.A are grateful to the Anillo Científico Tecnológico ACT1107. S.E.G.M. and T.P.A. acknowledge funding from Programa Basal PFB16 (PIA CONICYT) and Centro Interdisciplinario de Neurociencias de Valparaiso (ICM-Economia P09-022-F). P.H., M.B. M.J.R., and U.G.V. were supported by a bilateral, german-chilean academic exchange project DAAD project no. 56206483 / CONICYT project no. PCCI 044.

10:20am **BI-TuM8 MP-SPR New Characterization Method for Interactions and Ultrathin Films**, ***Annika Jokinen***, *N.M. Grangvist, W.M. Albers, J.W. Sadowski*, BioNavis, Finland

INTRODUCTION

Surface Plasmon Resonance (SPR) has been used already for a few decades for label-free detection and characterization of biochemical kinetics and affinities of many different types of analytes. The physical phenomenon is not limited to biochemistry, but is applicable to other nanoscale characterization of thin films[1].

EXPERIMENTAL METHODS

Aside of the traditional interactions, Multi Parametric Surface Plasmon Resonance (MP-SPR) can be utilized to determine unique refractive index (*RI*) and thickness (*d*) of ultrathin (d 0.5-100 nm) and slightly thicker films (d 300 nm- few µm) without prior assumptions of the RI of the material. These are important properties not only for thin film coating industries and applications, but also for gaining important knowledge in biomaterials. Two methods utilizing MP-SPR to thickness and RI calculations have been introduced, either measuring in two different media (2M) with high *RI* difference, such as air and water[1-3] , or at two or more different wavelengths (2W) of light[2,3] in order to characterize properties of the thin films.

RESULTS AND DISCUSSION

MP-SPR is suitable for film deposition *in situ* or *ex situ*, which makes it compatible with several deposition methods and thereby makes it applicable to a wide range of surfaces also. Polyelectrolyte multilayer deposition *in situ* was monitored in real-time with MP-SPR. Thickness of each deposited layers was determined utilizing two wavelength method.

Similarly layer thickness and RI was determined also for *ex situ* spin coated cellulose layer. MP-SPR was used not only to determine thickness and RI of the deposited layer but also for real time monitoring of other molecules interaction to the cellulose model surface[4,5].

Recently, MP-SPR was used also to monitor polymer layer structural changes in real time, such as polymer swelling due to pH or electric potential change[6]. At pH 9 poly (acrylic acid) (PAA) brushes were extended but the brushes collapsed at acidic pH[6].

CONCLUSION

With the ability to characterize both kinetics and nanoscale layer properties, MP-SPR proves to be a versatile tool for nanomaterial, biomaterial and biochemical interactions research, which makes MP-SPR invaluable for multidisciplinary research, where both physical and interaction properties of the materials need to be characterized.

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4. Orelma et al., 12 (12), 2011

5. Kontturi et al., J.Mater. Chem. A, 2013, (ASAP) DOI: 10.1039/C3TA12998E

6. Malmström et al., Macromolecules, 46 (12), 2013

10:40am **BI-TuM9 *In-Situ* Analysis of Biological and Electrochemical Interfaces Using ToF-SIMS**, ***Zihua Zhu***, *X. Yu, Z. Wang, B. Liu, X. Hua, L. Yang, M. Marshall, S. Thevuthasan, J. Cowin*, Pacific Northwest National Laboratory

In-situ analysis of liquid interfaces using ToF-SIMS is challenging because ToF-SIMS is a high-vacuum technique, but liquids often generate some considerable vapor pressure. For example, the vapor pressure of water is about 20 kPa at room temperature (20 °C), thus handling samples containing water in vacuum is not easily done. We recently developed a self-contained microfluidic device for probing aqueous surfaces and demonstrated its feasibility in ToF-SIMS and SEM.[1,2] The key feature of this device is a small round aperture with a diameter of 2-3 microns, which is opened on top of a microfluidic channel. The aperture is exposed to vacuum and serves as a detection window for ToF-SIMS measurements. Our calculations and experimental data show that vacuum compatibility and possible temperature drop due to water vaporization under vacuum can be well-controlled.Performance of the microfluidic device for *in situ* ToF-SIMS analysis of selected organic molecules at aqueous surfaces has been tested.[3] This new innovation has been used in *in-situ* study of mechanism of biofilm growth[4] and electrochemical reactions[5] that occur at liquid-solid interfaces. Such in-situ chemical information at liquid-solid interfaces is very difficult to be obtained using other techniques.

[1] L. Yang, X. Y. Yu, Z. Zhu, M. J. Iedema, J. P. Cowin, *Lab Chip*, **2011**, *11*, 2481-2484.

[2] L. Yang, X. Y. Yu, Z. Zhu, S. Thevuthasan, J. P. Cowin, *J. Vac. Sci. Technol. A*, **2011**, *29(6)*, 061101.

[3] L. Yang, Z. Zhu, X. Y. Yu, S. Thevuthasan, J. P. Cowin, *Anal. Methods*, **2013,** *5*, 2515.

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[5] B. Liu, X. Y. Yu, Z. Zhu, X. Hua, L. Yang, Z. Wang, *Lab Chip*, **2014**, *14*, 855.

11:00am **BI-TuM10 Quantifying ToF-SIMS Depth Profiles and 3D Images for Biological and Organic Materials**, *J. Taylor, D.J. Graham,* ***David Castner***, University of Washington, USA

To process, reconstruct, and understand the 3D data from complex materials such as multi-component polymers, drug delivery scaffolds, cells and tissues, it is essential to understand the sputtering behavior of these materials. Though much is understood about sputtering characteristics of some organic materials, there is still a general lack of understanding of how organic and biological materials sputter, especially as the complexity of the materials increase. For example, in multicomponent systems each component may have a different sputter rate, resulting in differential sputtering that will distort the reconstructed depth profile. Thus, accurate reconstruction involves accounting for differential sputter rates, complex sample geometries, etc. Polystyrene and PMMA on Si were used as model systems to optimize methods for depth profile reconstruction. Depth profiling of single component and bilayer films was performed using an Ar_{1000}^+ sputter source and Bi_3^+ analysis beam on an ION-TOF V ToF-SIMS instrument. PMMA sputtered at a significantly higher rate than polystyrene, whilst sputtering of Si can be considered negligible.

Typically the z-axis of depth profiles is converted to depth using an average sputter rate based on measured film thickness and time to remove the film. However, this fails to account for sputter rate variations during the profile, leading to inaccurate film thickness, interfacial position and resolution, and the appearance of penetration into the Si substrate. Applying measured single component sputter rates to the bilayer films, and assuming a step change in sputter rate, yields more accurate film thickness and interface positions; noticeably sharpening the polymer-Si interface. The conversion from sputter time to depth can be further improved by applying a linear change in sputter rate between components across the interface. This further sharpens the interfaces, bringing overall film thickness and interface position more closely in line with expected values. We also have observed a gradual change in sputter rate with mixed polymer blends, possibly due to nanoscale interfacial mixing during sample preparation and storage or induced during the sputter process. Sensitivity analysis performed on variables in sputter rate measurements reveals further scenarios for inaccurate depth profile reconstruction.

This work with a simple laminar system highlights the need for both careful evaluation of component sputter rates and correct application of methods for conversion of sputter time to depth if accurate 3-D reconstructions of complex multi-component samples such as tissue engineering scaffolds are to be achieved.

11:20am **BI-TuM11 *In Situ* Neutron Scattering Studies of Endothelial Cells Response to Shear Stress**, ***Jaroslaw(Jarek) Majewski***, *S. Junghans*, Los Alamos National Laboratory, *L. Pocivavsek,* University of Pittsburgh, *N. Zebda, G. Birukov*, University of Chicago

Neutron reflectivity is very well established experimental tool for obtaining length-scale and density information about well-ordered, layered materials of consistent thickness and high surface occupancy, such as *model* phospholipid bi- and mono-layers, polymeric thin films, inorganic layered structures, *etc.* It is much more difficult to obtain any information about poorly stratified samples and samples that incompletely cover the surface. Measuring *living cells* adhesion and response to external stimuli like the fluid (blood) flow provided an interesting challenge because of the complexity, disordered nature, inherent inhomogeneity of the system, a difficulty in controlling and producing samples with consistent surface coverage but also *biological safety requirements*. Despite these challenges, meaningful results can be obtained. I will discuss measurements involving adhesion of human endothelial cells under fluid mechanical shear stress [1]. Understanding of the cell adhesion in dynamic conditions is connected with pathologic buildup of lipids in arterial walls: atherosclerosis . Although

atherosclerosis is responsible for hundreds of thousands of deaths each year from heart attacks and strokes its nature is not fully understood.

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11:40am **BI-TuM12 3D Collagen-Based Biomaterials Assembly: Novel Insights from Advanced Optical Characterization**, *X. Lang, M. Spousta, J. Gigante, A. Vu, Y. Hwang,* ***Julia Lyubovitsky***, UCR

Optical methods are uniquely suited for characterization of complex biological systems due to their generally non-destructive nature. The applications include characterizing biomaterials/devices employed as medical implants or tissue engineered scaffolds. We have been developing advanced optical imaging guided spectroscopy methods to study the structures of 3D collagen-based biomaterials. This talk will summarize the novel insights regarding the physicochemical controls of assembly of collagen biopolymer into the fibers within 3D hydrogels, cross-linking, digestibility and quantification of hydrogels' structural parameters. For example, our recent study indicated that ions strongly affect the aggregation of collagen into the fibers and consequently modulate the length of the fibers that can be prepared. Changing the temperature led to a multilateral response that depended on the type of ion employed. The knowledge obtained can be applied to explore the practically important and complex processes during assembly and dis-assembly of collagen in engineering of functional biomaterials.

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