Biomaterial Interfaces
Room: 211D - Session BI-WeM

Biomolecules at Interfaces
Moderator: Axel Rosenhahn, Ruhr-University Bochum

8:00am **BI-WeM1 Understanding Hydration of Proteins by SALVI and Liquid ToF-SIMS**, ***Jiachao Yu***, *Y. Zhou, X. Hua, Z. Zhu,* Pacific Northwest National Laboratory, *S. Liu,* Southeast University, China, *X.-Y. Yu*, Pacific Northwest National Laboratory

Hydration is crucial to the structure, conformation, and biological activity of proteins. Proteins without water molecules surrounding them would not have viable biological activity. Specifically, water molecules will interact with the surface and internal structure of proteins, and different hydration states of proteins make such interactions distinct. Thus, it is important to understand the hydration of proteins on surfaces, which can provide a fundamental understanding of the mechanism of their structure, conformation, and biological activity. Our group developed an important technique to study liquid surfaces and interfaces, namely System for Analysis at the Liquid Vacuum Interface (SALVI). It has been recently applied to study hydrated protein biofilms. SALVI is a vacuum compatible microfluidic device that consists of a SiN window as the detection area and a microchannel made of polydimethylsiloxane (PDMS). The protein solution was introduced into the microchannel. After incubating for a period of time, a hydrated protein biofilm formed on the back side of the SiN membrane. The information of hydrated proteins was collected using the time-of-flight secondary ion mass spectrometry (ToF-SIMS) in the SALVI device in the liquid state. Compared with previous results from dry protein samples, we not only confirmed the amino acid compositions of proteins, but also firstly discovered that the distribution of water molecules surrounding and inside proteins were varied among different types of proteins. Our liquid ToF-SIMS results show that 1). The water clusters number and relative counts vary among the same hydrated proteins, which imply that the distribution of water molecules surrounding and inside a protein is inhomogeneous; 2). The same water clusters have varied content in different types of proteins, which indicate that the distribution of water molecules have a strong relationship with the structure and conformation of the proteins at the biointerface. These first observations of hydrated protein biofilms on a surface will pave the investigation of the structure, conformation, and biological activity of proteins in the future.

8:20am **BI-WeM2 Direct Measurement of the Interaction Free Energies of Single Hydrophobic Peptides with Extended Hydrophobic Surfaces**, ***Philipp Stock***, *T. Utzig, M. Valtiner*, Max-Planck Institut für Eisenforschung GmbH, Germany

Placing hydrophobes into an aqueous medium gives rise to what is well known as so-called hydrobobic interaction (HI) or hydrophobic force. This thermodynamic driven force gives rise to interaction and/or self-organisation of solvated hydrophobes in water. Famous examples are protein folding, lipid-bilayer membrane stability and enzymatic catalysis.

Here we will describe a SM-AFM setup to measure the interaction free energies (ΔG_0) of eight different hydrophobic peptides interacting with extended hydrophobic surfaces. First, we estimate the free energy of a single hydrophobic unit interacting with an extended hydrophobic surface. Secondly, we measured the change in free energy upon increasing the number of hydrophobic units in different sequences (synergies vs antagonistic effects).

In particular, we studied the change in free energy by placing a spacer groups (glycine) between the hydrophobic units. Interestingly our data shows that the interaction free energy scales with the number of hydrophobic units. Each hydrophobic unit seems to contribute about 4.8 kT. As such we observe a good agreement with values measured for two interacting benzene molecules in water.

Hence, hydrophobic interaction energies of hydrophobic surfaces with hydrophobic peptide fragments on a flexible peptide chain seem to linearly add up, irrespective of the incorporation sequences.

8:40am **BI-WeM3 Cells and Extracellular Matrices as Smart Materials: Dissecting and Rebuilding Mechaniobiological Units**, ***Sanjay Kumar***, University of California, Berkeley **INVITED**

Living cells are capable of processing a variety of mechanical signals encoded within their microenvironment, which can in turn act through the cellular structural machinery to regulate many fundamental behaviors. In this sense, cells may be regarded as "smart materials" that dynamically and locally modulate their physical properties in response to environmental stimuli. I will discuss our recent efforts to understand and control these living materials, and to create new, bio-inspired materials that mimic sequence/structure/function relationships of cytoskeletal networks. Key areas of emphasis will include: (1) Understanding and targeting biomechanical regulation of tumor infiltration in the brain; (2) Engineering stimulus-sensitive intrinsically disordered protein brushes based on neuronal cytoskeletal networks. These efforts exemplify the important notion that biomaterials can be extremely valuable platforms with which to understand and control cell behavior, and that understanding cellular structural networks can yield unexpected insights that inform the creation of novel biomaterials.

9:20am **BI-WeM5 Molecular Modeling of Biofunctionalized Hydrogels to Guide Hydrogel Design**, *X. Li,* Clemson University, *M.L. Becker,* University of Akron, *N.S. Murthy,* Rutgers University, ***Robert Latour***, Clemson University

Peptide-functionalized PEG-based hydrogels represent the workhorse material for tissue engineering and regenerative medicine applications because of their potential to mimic the extracellular matrix and serve as a substrate to direct cellular response. In order for a bioconjugated hydrogel to exhibit its intended bioactivity, the peptides that are tethered within the hydrogel must be accessible at the hydrogel surface for cell-receptor binding. Surface availability for a given peptide and hydrogel system will be a function of design parameters such as tether length, tether structure, and hydrogel crosslinking density. While the surface-accessibility of the peptide can be readily assessed experimentally, reasons for low accessibility (if encountered) are not easily determined. To address this type of problem, we are developing molecular modeling and simulation methods that will provide the ability to understand and visualize hydrogel behavior at an atomistic level to serve as a potentially powerful tool for hydrogel design. We are developing the molecular models using a multiscale approach. Coarse-grained (CG) parameters are first obtained from all-atom models of the various structural elements of the hydrogel system in aqueous solution using the polymer consistent force field (PCFF). A coarse-grained structure of the hydrogel is then first created at the experimental crosslink density using an efficient on-lattice method. The CG model is then removed from the lattice and equilibrated using the TIGER2 advanced sampling algorithm. The resulting equilibrated CG system is then reverse-mapped to an all-atom model. The all-atom model is then hydrated in water and equilibrated once more to yield the final predicted structure of the system. The resulting models are validated by comparing with structure-factor plots obtained by neutron scattering and/or X-ray diffraction. The resulting molecular models provide an atomistic-level view of peptide accessibility at the hydrogel surface. If low accessibility is encountered for a given design, the molecular models can provide clear direction regarding the cause of the problem and indicate the design changes that should be made to improve the bioactivity of the system.

9:40am **BI-WeM6 Physisorption of Stimuli-Responsive Polypeptides with Genetically Programmable Aqueous Phase Behavior**, ***Linying Li***, *C. Mo, Q. Tu, N.J. Carroll, A. Chilkoti, S. Zauscher,* Duke University, *M. Rubinstein,* University of North Carolina at Chapel Hill, *G.P. López*, Duke University

The ability to control the physical adsorption (physisorption) of proteins to solid surfaces is of fundamental importance in the design of engineered bio-interfaces for many biomaterial, industrial and bioanalytical applications. We present a study of the kinetics of adsorption and consequent single- and multi-layered architectures of recombinant, intrinsically disordered proteins whose aqueous phase behavior is programmable at the sequence level. Elastin-like polypeptides (ELPs) are a class of engineered repetitive polypeptides that undergo a reversible, lower critical solution temperature (LCST) phase transition in water. Their phase behavior is programmable by tuning the amino acid sequence, concentration, and molecular weight of the ELPs. We used light scattering assays to investigate the phase diagrams of the peptides and quartz crystal microbalance with energy dissipation (QCM-D) to investigate the diffusion-limited adsorption kinetics of ELPs onto surfaces. Below the critical temperature, ELPs are soluble and only form single monolayers of peptides on surfaces upon adsorption, while above the critical temperature, ELPs phase separate, leading to multilayer adsorption. We used ellipsometry and atomic force microscopy (AFM) to characterize the thickness and roughness of the protein assemblies on surfaces. The elemental composition of the protein-modified surfaces was analyzed by X-ray photoelectron spectroscopy (XPS). Contact angle measurements were performed to examine the temperature-responsive nature of the surfaces. This study demonstrates that, based on their genetically encoded phase behaviors, the adsorption behavior of ELPs can be controlled to attain

desired architecture, thermal-responsive behavior and functionality. It also provides insight into protein adsorption at the molecular level that can be useful in a number of contexts including immunoassays, drug delivery and cell culture.

11:00am **BI-WeM10 Evidence of a Molecular Boundary Lubricant at Snakeskin Surfaces**, ***Joe Baio***, Oregon State University, *M. Spinner*, University of Kiel, Germany, *C. Jaye, D.A. Fischer*, National Institute of Standards and Technology (NIST), *S. Gorb*, University of Kiel, Germany, *T. Weidner*, Max Planck Institute for Polymer Research, Germany

Snake scales have direct mechanical interaction with the environment. During slithering the ventral scales at a snake's belly are permanently in contact with the substrate, while the dorsal scales have an optical function for camouflage and thermoregulation. Recently it has been shown that ventral scales have adapted to this biological function and provide improved lubrication and wear protection compared with dorsal scales. While biomechanic adaption of snake motion to specific habitats is of growing interest in material science and robotics, the molecular level mechanism for the frictional influence of ventral scales is unknown. In this study, we characterize the outermost surface of snake scales using sum frequency generation (SFG) spectra and near edge x-ray absorption fine structure (NEXAFS) images collected from freshly molted California kingsnake (*Lampropeltis californiae*) scales. NEXAFS microscopy enables the mapping of specific molecular bonds at the C and N K-edges. The resulting NEXAFS images highlight the intensities of C=C π^*, σ^* (C−H), C=O π^*, and amide π^* bonds, demonstrating that the chemistry across the scale surfaces is uniform. SFG spectra at the amide I vibrational band (1550-1850 cm^{-1}) were collected from ventral and dorsal scales across three different individuals. Within the spectra taken from both types of scales, we observe a single peak at 1746 cm^{-1} that originates from ordered ester groups. In the CH stretching region, we observe two distinct vibrational modes in the spectra collected from the dorsal scale - 2850 and 2865 cm^{-1}. Both of these modes stem from symmetric CH_2 vibrations. Three bands are present in the CH spectra from the ventral scale - 2850, 2875, and a broad peak at 2975 cm^{-1}. Again, the peak at 2850 cm^{-1} is related to CH_2 symmetric vibrations, while the peaks at 2875 and 2975 cm^{-1} are related to symmetric and in plane anti-symmetric CH_3 stretches, respectively. Combined this analysis reveals the existence of a previously unknown lipid coating on the surfaces of both the ventral and dorsal scales with molecular structure closely related to their biological function: lipids on ventral scales form a highly ordered layer which provides both lubrication and wear protection at the snake's ventral surface.

11:20am **BI-WeM11 Selective Self-Assembly of Acidic Nanofibrils by a Calcite-Binding Barnacle Cement Protein**, *C. So*, National Research Council postdoc cited at Naval Research Laboratory, *J. Liu, K. Fears, D. Leary, J. Golden*, ***Kathryn Wahl***, US Naval Research Laboratory

Barnacles adhere by secreting a micron-thick proteinaceous layer between themselves and the marine environment that persists throughout their lifespan. These proteins play a dual role in adhering to both the native organism and a foreign substratum, which are often crystalline calcium carbonates from other marine invertebrates, cuticular exoskeleton or sedimentary minerals. Though the sequence and composition of several barnacle cement proteins have been reported, little is known about how these proteins become stably bound to surfaces. Here we use *in situ* atomic force microscopy (AFM) to examine a recombinantly expressed, acidic, calcite-binding 20kDa cement protein, MRCP20. We find that the protein immobilizes on the surface through recognition of distinct atomic steps on the [1014[face of calcite, further assembling on these features into stable nanofibrils. The protein fibrils are continuous and organized at the nanoscale, exhibiting striations with a period of *ca.* 45 nm. The acidic fibrils are also found to manipulate calcite surfaces through the dissolution of underlying calcite features that display the same atomic arrangement. To quantify selectivity, we compare the velocity of atomic steps from calcite etch pits when exposed to water, bulk protein solution, and surface-associated nanofibrils. MRCP20 is found to favor interaction with distinct fast moving steps, where velocity is increased by four- and eight-fold upon exposure to bulk proteins and fibrils, respectively, over steps exposed to solution without protein present. Calcite mineralized in the presence of MRCP20 results in asymmetric crystals, suggesting a similar step-selective behavior by MRCP20 during crystal growth. Cooperative molecular processes with step edge atoms reveal a new regime of biotic interactions with calcite, where specific surface interactions are enhanced through templated long-range nanostructures.

11:40am **BI-WeM12 Thiolene Reaction Applied to Passive Metal Oxide Surfaces for Addressing Protein Adsorption and Cell Adhesion**, ***Anouk Galtayries***, *V. Semetey, A. Dellinger*, Chimie ParisTech, France

The aim of this work is to design surfaces allowing controlling biomolecule adhesion by the study of protein adsorption and cell adhesion. In order to answer this challenge,: the optimization of grafting conditions using the thiol-ene reaction of thiol-terminated ethylene glycol (EG) chains (Oligo-EG or Poly-EG) on a undecenyltrichlorosilane self-assembled monolayer was investigated [1], with the help of surface characterization (angle contact measurement, ellipsometry, fluorescence microscopy, attenuated total reflection infrared *IR-ATR*, X-ray Photoelectron Spectroscopy *XPS*, Time-of-Flight Secondary Ion Mass Spectrometry *ToF-SIMS*) after each reaction step.

Varying different reaction parameters in the methodological investigation of thiol-ene grafting conditions exhibits the development of a bilayer structured system after a 1 minute reaction time as regards OEG grafting, and 1 hour time for PEG grafting. By using different passivated substrates (model silicon single crystal, polycrystalline titanium), different OEG-thiol or PEG-thiol molecules (from 7 to 220 ethylene unit long, methyl-, carboxyl- or amine-terminated), we highlight the range of available versions of this strategy. The terminal chemical functions lead on demand either to protein adsorption inhibition or to biomolecule adsorption, bovine serum albumin (BSA) or fibronectin (Fn) giving access to specific adhesion.

By controlling the light-exposed areas (100 mm-large bands or half-moon surfaces), the photochemistry occurring during the thiol-ene grafting allows to design surface patterning for addressing both protein adsorption and cell adhesion on such sample biointerface on metal oxides.

[1] ``A Facile and Versatile Approach to Design Self-Assembled Monolayers on Glass using Thiol-ene Chemistry", B. Oberleitner, A. Dellinger, M. Déforet, A. Galtayries, A.-S. Castanet,V. Semetey, Chemical Communication, 49, 1615-1617 (2013).

12:00pm **BI-WeM13 Scaling from Single Molecule to Macroscopic Adhesion at Polymer/Metal Interfaces**, ***Thomas Utzig***, *S. Raman, M. Valtiner*, Max-Planck Institut für Eisenforschung GmbH, Germany

Understanding the evolution of macroscopic adhesion based on the fundamental molecular interactions is crucial to design strong and smart polymer/metal interfaces, which play an important role in many industrial and bio-medical applications. Here we show how macroscopic adhesion can be predicted based on single molecular interactions. In particular, we carry out dynamic single molecule force spectroscopy (SM-AFM) in the framework of Bell-Evans' theory to gain information about the energy barrier between the bound and unbound state of an amine/gold junction. Further we use Jarzynski's equality to obtain the equilibrium ground state energy difference of the amine/gold bond from these non-equilibrium force measurements. In addition, we perform Surface Forces Apparatus (SFA) experiments to measure macroscopic adhesion forces at contacts where approximately 10^7 amine/gold bonds are formed simultaneously. The SFA approach provides an amine/gold interaction energy (normalized by the number of interacting molecules) of 36 ± 1 k_BT, which is in excellent agreement with the interaction free energy of 35 ± 3 k_BT calculated using Jarzynski's equality and single molecule AFM experiments. Our results validate Jarzynski's equality for the field of polymer/metal interactions by measuring both sides of the equation. Furthermore, the comparison of SFA and AFM shows how macroscopic interaction energies can be predicted based on single molecular interactions, providing a new strategy to potentially predict adhesive properties of novel glues or coatings as well as bio- and wet adhesion.

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