Biomaterial Interfaces
Room: 317 - Session BI+AS-MoM

Biomolecules & Biomaterials Interfaces
Moderator: Ilya Reviakine, CIC biomaGUNE

8:40am **BI+AS-MoM2 Deposition of Porous Polyparylene Layers with Even Thickness in Narrow Tubes**, ***Gerhard Franz***, *H. Heidari*, Munich University of Applied Sciences, Germany

To coat a thin hollow tube with an equally thick layer along the whole length, is one of the most challenging issues of surface refinement. Even for long mean free paths and large diffusion lengths, a drop in thickness is common, which is simply caused by the abstraction of deposited molecules, which cannot walk randomly any longer. To overcome these inherent spatial inhomogeneities, we made use of the mechanism of the temperature-dependent surface polymerization, which is manifested in the occurence of a "ceiling temperature". Negatively turned, no deposition is possible beyond this temperature. Positively spoken, the spatially inhomogeneous deposition rate along a tube can be equalized with a counteracting temperature gradient. Experimentally, a configuration with four furnaces in line has been constructed which allows the inner wall of a tube 12" in length and 1/8" in inner diameter to be coated with a layer of even thickness. The most prominent application is the partial protection of thin silver layers which are deposited on the inner walls of catheters of polyurethane or polysilicone not as a contiguous film but with a zebra-stripe design applying a patent-pending procedure [1]. These silver rings act as antibacterial means to combat infections and induced incrustations in the urological area. To prolong the lifetime of the silver depot, it has to be protected with a porous human compatible top layer. We chose FDA approved polyparylene with thicknesses between 100 and 400 nm to ensure a long-term antibacterial activity, which should be kept above threshold level by a safety factor of 2 [2,3]. First results for the CVD of polyparylene are presented and are discussed and modeled with COMSOL in terms of diffusion laws with an abstraction reaction of 1st order. After having shown the antibacterial effect for a static case [4], here a dynamic trial is presented to simulate the antibacterial activity during flow of bacteria-containing urine in the ureters. [1] G. Franz, F. Schamberger, A. Kutschera, S. Seyedi, D. Jocham, German patent disclosure DE 102012023349.3, Nov. 29, 2012, [2] F. Schamberger, A. Ziegler, and G. Franz, J. Vac. Sci. Technol. B30, 01801 (2012) [3] G. Franz, F. Schamberger, J. Vac. Sci. Technol. A31, 061602 (2013) [4] H. Heidari, St. Sudhop, F. Schamberger, G. Franz, Biointerphases, accepted May 05, 2014

9:00am **BI+AS-MoM3 Deciphering the Scaling of Single Molecule Acid-Amine Interactions using Jarzynski's Equality**, *S. Raman, T. Utzig, T. Baimpos, B.R. Shrestha,* ***Markus Valtiner***, Max Planck Institut fur Eisenforschung GmbH, Germany

Unraveling the complexities of the macroscopic world based on molecular level details relies on understanding the scaling of single molecular interactions towards integral interactions, which are mediated through a large number of simultaneously interacting molecular bonds. Here we demonstrate how to decipher the scaling of acid-amine interactions from the single molecular level towards the macroscopic level through a synergistic experimental approach combining equilibrium Surface Forces Apparatus (SFA) experiments and non-equilibrium single molecule force spectroscopy (SM-AFM). Combining these two techniques is ideally suited for testing the largely praised Jarzynski's equality (JE), which relates the work performed under non-equilibrium conditions with the equilibrium free energy. Large-scale equilibrium force measurements using SFA scale linearly with the number density of acid-base bonds at an interface and we measure molecular acid-amine interaction energies of 10.9 ± 0.2 kT. AFM single molecule experiments reveal two distinct regimes. As expected, far from equilibrium the measured single molecule unbinding forces increase exponentially with the loading rate. A second quasi-equilibrium regime at loading rates close to and below the natural binding/unbinding rate of the acid-amine bond shows little loading rate dependence. Irrespective of how far from equilibrium AFM experiments are performed, the energy calculated using JE converges rapidly to 10.7 ± 1.1 kT. This is essentially equivalent to the value measured by the equilibrium measurements using SFA. Our results suggest that using Jarzynski's equality allows direct scaling of non-equilibrium single molecule interaction force measurements to scenarios where a large number of molecules are simultaneously interacting, giving rise to macroscopic equilibrated interaction energies. Taken together, the developed approach provides a strategy for molecular design of novel functional materials through predicting of large-scale properties such as adhesion or cell-substrate interactions based on single molecule or simulation experiments.

9:20am **BI+AS-MoM4 Fabrication of ssDNA Monolayers, Custom Designed ssDNA Arrays and Brush Patterns in Biorepulsive Templates by Promoted Exchange Reaction**, *M.N. Khan,* University of Heidelberg, Germany, *V. Tjong, A. Chilkoti,* Duke University, ***Michael Zharnikov***, University of Heidelberg, Germany

We present here a versatile approach to prepare mixed monolayers of thiolate-bound single stranded DNA (ssDNA) and oligo(ethylene glycol) substituted alkanethiols (OEG-AT) in a broad range of compositions as well as ssDNA/OEG-AT patterns of desired shape embedded into a biorepulsive background. The procedure involves two steps. First, a OEG-AT monolayer on a solid support is exposed to electrons or UV light in either homogeneous or lithographic fashion. Second, the promoted (by the irradiation in the first step) exchange reaction between the damaged OEG-AT species in the film and ssDNA substituents in solution occurs, resulting in formation of a ssDNA/OEG-AT monolayer or pattern. The composition of the mixed films or ssDNA/OEG-AT spots (lithography) can be precisely adjusted by electron or UV dose in almost entire composition range. The above procedure relies on commercially available compounds and is applicable to both thiol-terminated and symmetric and asymmetric disulfide-terminated ssDNA. The fabricated OEG-AT/ssDNA templates and patterns can be extended into the z-dimension by surface-initiated enzymatic polymerization of ssDNA, which results in the formation of highly ordered ssDNA brushes and allows topographically complex ssDNA brush patterns to be sculpted on the surface.

9:40am **BI+AS-MoM5 High Throughput BioMaterials Screening using Microarrays and High Information Content Imaging Methods**, *S. Boudjabi, D. Covelli, M. Keramane, E. Luckham,* ***John Brennan***, McMaster University, Canada **INVITED**

This presentation will highlight recent work in the area of high throughput screening of biologically modified surfaces for production of biosensors, protein and cell microarrays, and non-fouling surfaces. Using robotic material synthesis and assay systems and a combination of contact and noncontact microarray printing, we have produced several libraries of biomaterials with a wide range of chemical compositions based on acrylate, silicone and silica-based polymers. Using silica-based materials as an example, the presentation will show the workflow utilized to develop new bioactive polymer materials for generation of bioactive and stealth materials and coatings. This includes methods to produce several thousand materials very rapidly via printing, rapid imaging tools and assays for screening to identify "hits" that show a desired property (i.e., high bioactivity, low non-specific binding), and methods for detailed material analysis using a range of imaging methods based on fluorescence, XPS, MALDI-MS/MS, FTIR and SPR to fully characterize the properties of biologically active materials. Methods for mining and analyzing the large datasets produced using our in-house developed Biointerfaces Research Gateway will be described.

10:40am **BI+AS-MoM8 Osteocalcin Adsorption onto Calcium Phosphate and Silica Surfaces**, *L.A. Scudeller,* ***David Castner***, University of Washington

Osteocalcin (OC) is the most abundant, non-collagenous protein in bone and accounts for almost 2% of total protein in the human body. OC plays a role in the body's metabolic regulation and bone building, as well as being used as a biochemical marker for bone formation. However, its precise function is not known. OC is known to bind strongly to hydroxyapatite (HAP). This strong binding is likely the result of the γ-carboxylated glutamic acid residues (Gla) in OC interacting with Ca^{2+} ions on the HAP surface. OC has three helical units (α-1, α-2 and α-3) and the spacing of the 3 Gla residues in the α-1 unit match well the lattice spacing of the (001) HAP surface.

This study uses x-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) to investigate the adsorption of OC and decarboxylated (i.e., Gla converted back to Glu) OC (dOC) onto various calcium phosphate surfaces as well as silica surfaces. The XPS nitrogen signal is used to track the amount of adsorbed OC and dOC. The intensities of key ToF-SIMS amino acid fragments are used to assess changes in the structure of adsorbed OC and dOC.

The largest differences were observed between OC and dOC adsorbed onto the silica and HAP surfaces. Similar amounts (3-4 atomic % N) of OC and dOC were adsorbed onto the silica surface. Higher amounts adsorbed on the HAP surface (~5 atomic % N for dOC and ~8 atomic % N for OC). The ToF-SIMS data showed the intensity of the Cys amino acid fragment, normalized to intensity of all amino acid fragments, was significantly higher

(~x10) when the proteins were adsorbed onto silica. Since in the native OC structure the cysteines are buried in the center of the 3 α-helices, this indicates both OC and dOC are more denatured on the silica surface. As OC and dOC denature upon adsorption to the silica surface the cysteines become more exposed and are more readily detected by ToF-SIMS. No significant differences were detected between OC and dOC adsorbed onto the silica surface, but small differences were observed between OC and dOC adsorbed onto the HAP surface. In the OC structure the α-3 helix is located above the α-1 and α-2 helices. Small differences in the ToF-SIMS intensities from amino acid fragments characteristic of each helical unit (Asn for α-1; His for α-2; and Phe for α-3) suggests either slight changes in the orientation or a slight uncovering of the α-1 and α-2 for adsorbed dOC.

XPS showed similar amounts of OC and dOC were absorbed onto amorphous HAP, crystalline HAP and octacalcium phosphate, but ToF-SIMS detected some small differences in the amino acid fragment intensities between adsorbed OC and dOC.

11:00am **BI+AS-MoM9 Reversible Activation of a pH-sensitive Cell Penetrating Peptides Attached to Gold Surfaces**, ***Joe Baio***, Oregon State University, *D. Schach,* University of Chicago, *M. Bonn, T. Weidner*, Max Planck Institute for Polymer Research, Germany

GALA peptides (WEAALAEALAEALAEHLAEALAEALEALAA) mimic pH-sensitive viral fusion proteins and are widely touted as a promising route to achieve site-specific delivery of therapeutic compounds. At basic pH, GALA assumes a random coil structure but when lowering the pH to acidic conditions the peptide transitions into an alpha helical structure. In this state, GALA has the ability to penetrate cell membranes and form pores. This mechanism is mainly driven by the change in overall charge of the glutamic acid side chains. One development of GALA mediated drug delivery is the immobilization of these peptides onto Au nanoparticles. Here we demonstrate, using a variety of spectroscopic techniques, that GALA can self-assemble into a protein monolayer on a gold film, linked to the surface via a single cysteine synthesized to the carbonyl terminus. Transmission IR vibrational spectroscopy demonstrates that the addition of this cysteine does not impede the pH transition between a helix and random coil structure in solution. Detailed characterization of the thiol-Au immobilization scheme by X-ray photoelectron spectroscopy illustrates that this single cysteine induced the formation of a well-ordered protein monolayer. To directly observe any pH triggered transition of this protein monolayer, sum frequency generation (SFG) vibrational spectra, at the amide I vibrational band, were collected at four different pH environments. A vibration mode at 1655 cm^{-1}, related to a helical structure, appears when this monolayer is immersed in a buffer at acidic conditions (pH 3 and 5) and then disappears under basic conditions (pH 9 and 12). While the surface immobilization clearly reduces the effective glutamic acid pK_a from a bulk solution value of 6 to 5.5, the covalently bound GALA-cysteine monolayer reliably retained the reversible, pH-driven helix-coil transition mechanism. Our findings establish that covalent attachment of GALA via cysteine linkers is a promising route for drug delivery applications and the design of 'smart' biological coatings.

11:20am **BI+AS-MoM10 Polydopamine Modification Using Small Molecule Thiols and Dithiols: Problems and Solutions for Creating Protein Resistant Coatings**, ***Marlon Walker***, *A. Vaish, D. Vanderah*, National Institute of Standards and Technology (NIST)

Polydopamine (PDA) is emerging as an increasingly useful bio-inspired coating for surface modification. Generated by a condensation reaction of dopamine in aqueous media under alkaline conditions, it can be readily deposited on almost any surface, forming thin films of controllable thicknesses. One useful attribute of a PDA coating is that it can be placed on and further modified to exhibit desired properties not possible with the underlying substrate. We present results of functionalizing PDA-coated surfaces on substrates such as silicon with oligo (ethylene oxide) thiols and dithiols for non-specific protein adsorption resistance.

11:40am **BI+AS-MoM11 A Process to Functionalize Polyaniline for Biotin-Avidin Biosensing**, ***Tiana Shaw***, *M.D. Williams*, Clark Atlanta University

Biotin-avidin technology is a widely explored interaction in bioscience. Biotin's affinity for the protein avidin, makes it ideal for protein and nucleic acid detection or purification methods. This strong interaction if often used in pretargeting strategies for cancer treatment. In most cases a probe molecule (antibody) is connected to a marker molecule (fluorophore or nanoparticle) through the biotin-avidin bridge. Biotinylated nanoparticles can play a role in improving this interaction and creating an electronic or optical detection method. Polyaniline is a polymer which can be easily functionalized to be specific for various biomolecules and has ideal sensor characteristics. In this study we will design a process to functionalize polyaniline with biotin to create a biotin-avidin biosensor. We began with 2-acetamidophenol which is a hydroxyl substituted aniline monomer. This monomer undergoes polymerization to yield 2-hydroxy polyaniline. The polymer's hydroxyl group was functionalized by Steglich esterification which refluxes a carboxylic acid with an alcohol. This esterification drives the reaction and dehydrates the products shifting the equilibrium towards the product. In this reaction DCC (dicyclohexylcarbodiimide) activates the carboxylic acid of biotin to further reaction and DMAP (4-dimethlyaminopyridine) acts as the acyl transfer catalyst. The biotinylated polyaniline derivative was characterized using FT-IR spectroscopy, ^{1}H NMR spectroscopy, UV-VIS spectroscopy, and Scanning Electron Microscopy. Florescence emission studies were also carried out with the avidin protein.

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