Biomaterial Interfaces
Room 318/319 - Session BI-ThM

Biosensors
Moderator: M. Tarlov, National Institute of Standards and Technology

9:00am **BI-ThM3 Simultaneous Electrochemical and Tapping Mode Imaging of Soft Biological Samples with AFM Tip Integrated Nanoelectrodes and Nanobiosensors**, ***A. Kueng***, *C. Kranz,* Georgia Institute of Technology*; A. Lugstein, E. Bertagnolli,* Vienna University of Technology, Austria*; B. Mizaikoff,* Georgia Institute of Technology

Recent developments in combined scanning probe techniques are aiming at complementary, simultaneously mapped information on physical and chemical surface properties with high spatial resolution. The integration of micro and nanoelectrodes into AFM tips using micromachining and focused ion beam (FIB) techniques recently described by our research group@footnote 1,2@ enables to simultaneously obtain laterally resolved electrochemical information at the sample surface during tapping mode AFM imaging. A defined geometry of an electroactive surface integrated above the very end of the original AFM tip allows direct correlation of the current signal and the topographical information. Hence, the functionality of scanning electrochemical microscopy (SECM) can be integrated into AFM. The presented technology enables integration of potentiometric electrodes or micro-/nanobiosensors providing simultaneous in-situ information on bioactive processes at the sample surface during AFM imaging. Due to the achieved current-independent positioning of the integrated electrode, biosensor functionality can be realized by modification of the electrode surface with an enzyme receptor, such as peroxidase or glucoseoxidase. Furthermore, bifunctional probes are applied to simultaneously image topographical and electrochemical properties of biologically active sample surfaces in AFM tapping mode. The activity of an oxidoreductase immobilized into a periodic micro-pattern of a soft polymer matrix is electrochemically detected during AFM imaging in tapping mode. For the first time specific detection of a molecular product resulting from enzymatic substrate conversion was obtained during AFM imaging. @FootnoteText@ @footnote 1@ C. Kranz, G. Friedbacher, B. Mizaikoff, A. Lugstein, J. Smoliner, E. Bertagnolli, Anal. Chem., 73, 2491-2500 (2001). @footnote 2@ A. Kueng, C. Kranz, B. Mizaikoff, A. Lugstein, E. Bertagnolli, Appl. Phys. Lett., 82, 1592-1594 (2003).

9:20am **BI-ThM4 Simultaneous Atomic Force Microscopy and Fluorescence Imaging of Supported Biomembranes**, ***A.R. Burns***, *J.M. Gaudioso,* Sandia National Laboratories

Lateral organization of lipids and proteins in membranes is critical to cellular signaling processes. Separately, fluorescence imaging and atomic force microscopy (AFM) are both effective ways to map structures in supported membranes. However, the ability to correlate information gathered from fluorescence imaging of labeled biomolecules and lipids with detailed lateral structures mapped out with AFM is highly advantageous. We discuss simultaneous AFM and submicron confocal fluorescence imaging of domain structures in model lipid bilayers. Lipids labeled by fluorescent probes either at the headgroups or tailgroups enable domain contrast in fluorescence imaging on the basis of partitioning between gel and disordered liquid phases. However, correlation with AFM topographic information reveals that they do not always faithfully report exact gel domain size or shape. Furthermore, we find that the fluorescence contrast decreases significantly with domain size, such that small domains observed with AFM are not observed in fluorescence images despite adequate optical resolution. Imaging of labeled proteins bound to membrane receptors is also discussed. In all cases, the complete correlation of topographic and fluorescence images provides evidence that gel-phase domains occur across both leaflets of the bilayer. This research was supported in part by the Division of Materials Science and Engineering, Office of Basic Energy Sciences, U.S. Department of Energy. Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the U.S. Department of Energy under Contract DE-AC04-94AL85000.

9:40am **BI-ThM5 Force Spectroscopy Investigation of HIV Envelope Glycoprotein and Dual Antibody Complex using Atomic Force Microscopy**, ***Y. Lam***, Duke Univ. and Center for Biomolecular and Tissue Eng.*; W.K. Lee,* Duke Univ.*; P. Marszalek,* Duke Univ. and Center for Biomolecular and Tissue Eng.*; M. Alam,* Human Vaccine Institute*; R. Clark,* Duke Univ.*; B. Haynes,* Human Vaccine Institute*; S. Zauscher,* Duke Univ. and Center for Biomolecular and Tissue Eng.

Understanding the structure-function relationships of pathogenic molecules is the key to designing sensitive detection mechanisms, as well as effective inhibitory drugs. Atomic force microscopy (AFM) is an optimal tool for investigation of these molecular scale biomechanics, as it provides high temporal and spatial resolution while maintaining an aqueous testing environment. In this study, we use the AFM to examine a well-characterized model system of Human Immunodeficiency Virus-1 (HIV-1) envelope glycoprotein gp120 and several monoclonal antibodies. Antibodies were screened before AFM experiments using surface plasmon resonance (SPR), and chosen for greatest binding affinities. In the AFM experiment, one antibody, a human T-cell CD4 mimic, is immobilized on the surface, and functions to bind gp120 from solution. The second antibody, a human chemokine receptor mimic, is attached to the AFM tip, and only interacts with the bound gp120-CD4 complex. A large majority of trials registered adhesion events, qualitatively signifying the presence of gp120. Quantitative analysis determined antibody-antigen binding strengths on the order of 100 pN, in agreement with binding forces of other molecular recognition systems. Experiments with poly(ethylene glycol) tethers and variable pulling rates provide force profiles revealing details of attachment and detachment mechanisms. Results from this study show that AFM can be used effectively as a detection as well as characterization method to better understand the pathogenic system.

10:00am **BI-ThM6 Multi-technique Studies of Bio-Interface Processes; QCM-D, (Nanoparticle) SPR, SERS, AFM, Electrical Impedance, and Cell Force Sensor**, ***B. Kasemo***, Chalmers University of Technology and Goteborg University, Sweden **INVITED**

Title: Multi-technique studies of Bio-Interface kinetics with QCM-D,(nanoparticle) SPR, SERS, AFM, Electrical Impedance, and Cell Force Sensor. Text: Bio-interface sensing is commonly based on immobilization of some sensing molecules on a surface, and detection of how unknown sample molecules bind specifically to them. Techniques for detection include optical, electrical, mechanical and electro-acoustic methods. The first part of this talk describes the preparation of a sensing platform based on functionalized supported phospholipid bilayers, using QCM-D, AFM, SPR and electrical impedance (ac forward transmission factor).@footnote 1, 2@ Sensing applications with this platform are illustrated by several examples;@footnote 3@ DNA immobilization and hybridization, protein-bilayer interactions, and enzymatic reactions. With the QCM-D technique rich information is obtained through frequency and dissipation shift measurements at multiple (overtone) frequencies, additionally strengthened by combining this technique with SPR. The second part describes nano- and micro fabricated structures for sensing, including EBL fabricated Ag arrays for (G)SERS,@footnote 4@ colloidal lithography of nanoparticles for plasmon resonance enhancement,@footnote 5@ and standing cantilever arrays for cell force sensing.@footnote 6@ @FootnoteText@ @footnote 1@ Reimhult, E., et al., Langmuir 19 (2003) 1681-1691. @footnote 2@ Hook, F., et al., Analytical Chemistry 73 (2001) 5796-5804@footnote 3@ Hook, F., et al., Langmuir 17 (2001) 8305-8312. @footnote 4@ Gunnarsson, L., et al., NanoStructured Materials 12 (1999) 783-788.@footnote 5@ Hanarp, P., et al., Colloids and Surfaces A: Physicochemical and Engineering Aspects 214 (2003) 23-26@footnote6@ Petronis, S. et al Journal of Micromechanics and Microengineering (to be published).

10:40am **BI-ThM8 Coupling of his-tagged scFvs to Functionalized Lipid Assemblies for Array Based Sensing**, ***C. Larsson***, *F. Höök,* Chalmers University of Technology, Sweden

Lipid bilayers containing 5% NTA-lipids supported on SiO@sub 2@ have been used as a template for efficient immobilization of oligohistidine-tag containing single-chained antibody fragments (scFv) directed towards cholera toxin (CT). It was demonstrated that his-tagged scFv:s is equally efficient coupled to the NTA/Ni@super 2+@ containing lipid bilayer from a purified sample and the expression supernatant. Using the latter, time consuming protein purification steps is avoided. Independent on whether the coupling was made from the supernatant or from the purified sample, the template was proven efficient for antigen detection, in this case verified via the quartz crystal microbalance with dissipation monitoring

(QCM-D) technique using the antigen CT (Mw ~85 kD). Via a secondary amplification step utilizing G@sub M1@ containing vesicles, i.e. the membrane receptor for CT, sub-nanomolar concentrations of CT was detectable with QCM-D. Furthermore, this coupling strategy was also utilized for creation of protein array templates. The template was, however, based on novel DNA-array design, using streptavidin-based DNA-immobilization on gold spots, surrounded by a pure lipid bilayer on SiO@sub 2@, but with the aim to be used as a protein array rather than a DNA array. The latter was accomplished using DNA-modified lipid vesicles, directed to predefined DNA spots via complementary hybridization, where the protein-array concept was proven utilizing scFv-modified lipid vesicles utilizing NTA/Ni@super 2+@-based coupling for highly sensitive detection of fluorescently labeled CT.

11:00am **BI-ThM9 Real-Time, Quantitative Surface Plasmon Microscopy Measurements of Protein Adsorption**, ***J.S. Shumaker-Parry***, University of Washington, presently at the Max Planck Institute for Polymer Research, Germany*; M.H. Zareie, C.T. Campbell,* University of Washington

Surface plasmon resonance (SPR) spectroscopy has become a popular technique for measuring biomolecular interactions in real time with high sensitivity and without labels. SPR microscopy provides the same advantages as SPR spectroscopy with the added feature of using a CCD camera to image changes in reflected light intensity across a large area of a sensor surface simultaneously with good spatial resolution (~ 4 μm). Recently we have developed quantitative SPR microscopy methods for measurement of adsorption and desorption processes in real time based on monitoring changes in reflected intensity at a high contrast angle. For a small range of angles in a linear region of a SPR curve, reflectivity changes are proportional to effective refractive index changes near the sensor surface. By fixing the angle of measurement at a high contrast angle in such a linear region, refractive index changes may be monitored in real time by measuring reflectivity changes for pre-selected regions of a sensor surface. By extending methods used to quantitate SPR spectroscopy wavelength and angle shifts to changes in reflectivity measured by SPR microscopy, quantitative, real-time adsorption measurements are possible. We used these methods to measure adsorption of a DNA-binding protein to its DNA-binding site immobilized in a double-stranded DNA (dsDNA) array on a streptavidin linker layer to demonstrate the use of SPR microscopy for parallel, high-throughput array-based analysis. A major advantage of these array-based studies is the ability to use array elements without the DNA-binding site as reference regions to correct for non-specific adsorption and common refractive index changes. The real-time detection limit for fast time resolution measurements is less than ~8 x 10@super 6@ proteins per 200-μm array spot.

11:20am **BI-ThM10 Material and Surface Characterization of Electrodeposited Polysaccharide Chitosan Film as a Platform for Biomolecular Reactions in BioMEMS Systems**, ***C. Pederzolli***, *L. Lunelli, G. Speranza, R. Canteri, M. Anderle,* ITC-IRST, Italy*; J.J. Park, L.-Q. Wu, H. Yi, R. Ghodssi, W.E. Bentley, G.F. Payne, G.W. Rubloff,* University of Maryland

The polysaccharide chitosan provides a high density of amine sites for biomolecular adsorption and reaction. Exploiting the fact that under mildly acidic conditions (pKa=6.3) chitosan is water soluble with the amine groups positively charged, we have electrodeposited chitosan onto negative electrodes and demonstrated schemes for attachment of fluorophores, proteins and nucleic acids. The chitosan films can be deposited as relatively compact films or hydrogels, depending on process conditions. We have studied film properties by changing to high pH after deposition, rinsing, and drying. Measurements indicate a complex structure with density substantially lower than expected for a closed packed film, consistent with AFM images which reveal a morphology with substantial (50-150 nm) roughness. One may anticipate deprotonation and local enhancement of the pH at the surface during deposition, which could be accompanied by H@sub2@ evolution; localized features observed in AFM images could be consistent with H@sub2@ bubble formation during electrodeposition. Estimates of amine site density made from these observations are in agreement with fluorescence intensity measurements that directly reveal densities in the range 10@super14@-10@super15@ sites/cm@super2@. XPS and ToF-SIMS reveal chemical components of glucosamine (-CH@sub2@-NH@sub2@) and also of N-acetylglucosamine (-NH-(C=O)-) the monomeric residues of chitosan. The extent of cell adhesion on these chitosan films was analyzed using fibroblast-like cells (NIH-3T3, HGF-1), and results indicate that cell adhesion and growth are dependent on deposition parameters and film thickness. These observations of the materials and surface properties are important for our use of electrodeposited chitosan as a platform for biomolecular reactions in bioMEMS systems.

11:40am **BI-ThM11 Infrared and Visible Spectroscopy of Anisotropic Spin-cast Chitosan Films**, ***W.H. Nosal***, *S. Sarkar, A. Subramanian, D.W. Thompson, J.A. Woollam,* University of Nebraska, Lincoln

Chemical modification of chitosan is postulated as a way to control thrombosis at the surface of biomedical implants. Recent interest in performing surface modification of biomaterials has led to chitosan as a candidate to improve biocompatibility, due to the relative ease with which the amine groups can be chemically modified. As a way to evaluate surface modification effectiveness, spin-cast films of chitosan on silicon wafers were characterized using both infrared and visible spectroscopic ellipsometry (SE). Infrared SE data was modeled using a set of harmonic oscillators to represent the chemical bonds of the chitosan molecule. Visible SE data was modeled using an anisotropic uniaxial Cauchy dispersion model. In-plane vs. Out-of-plane optical anisotropy was detected in both the Infrared and visible wavelength data. This work shows there is a preferred orientation of the molecule, likely a result of spin casting. Visible ellipsometry surface mapping was used to determine anisotropy as a function of position on the wafer. This work discusses anisotropy and orientation of identified chemical bonds in a biomolecular film by spectroscopic ellipsometry.

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