Biomaterial Interfaces Group
Room 613/614 - Session BI-MoM

Biosensor-Biology Interface

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

8:20am **BI-MoM1 TOF-SIMS Investigation of the Immobilization Process of PNA and DNA Biosensor Chips**, ***H.F. Arlinghaus***, *C. Höppener, J. Drexler, M. Ostrop,* Physikalisches Institut der Universität Münster, Germany

A novel DNA sequencing method is described that uses peptide nucleic acid (PNA) hybridization biosensor chips. PNA is a synthesized DNA analog, in which both the phosphate and the deoxyribose of the DNA backbone are replaced by polyamides. This DNA analog retains the ability to hybridize with complementary DNA or RNA sequences. Because the backbone of DNA contains phosphates, of which PNA is free, an analysis technique that identifies the presence of phosphates in a molecular surface layer allows the use of unlabeled DNA for hybridization on a biosensor chip. We used TOF-SIMS to investigate its ability to distinguished between PNA and DNA molecules on surfaces, as well as the PNA and DNA immobilization process. For this purpose we immobilized silane SA-layers on UV/ozone treated silicon wafers and bonded PNA and DNA with different concentrations to these layers. It was found that the immobilization process is strongly dependent on the concentration and the immobilization time and that under optimized conditions, PNA and DNA can be covalently bonded to the silane SA-layers. A comparison between positive and negative TOF-SIMS spectra showed that the masses corresponding to PO@sub 2@@super -@, PO@sub 3@@super -@ and H@sub 2@PO@sub 4@@super -@ provide the best correlation to DNA presence. The phosphate yield could be significantly increased with polyatomic ion bombardment. Temperature-programmed SIMS (TP-SIMS) was used to measure the thermal stability of the immobilized layers showing that characteristic silane fragment ions decrease at a temperature of about 70°C. It can be concluded that the combination of TOF-SIMS and TP-SIMS provides a very useful technique for examining the complexity of the immobilization and hybridization processes of nucleic acid and that TOF-SIMS has the potential for providing a rapid method for DNA/RNA sequencing and diagnostics.

8:40am **BI-MoM2 Characterization and Quantitation of DNA on Gold**, ***A.B. Steel***, Gene Logic, Inc.*; R.L. Levicky,* Columbia University*; T.M. Herne, M.J. Tarlov,* National Institute of Standards and Technology

The interaction of DNA with gold has been characterized using a number of analytical techniques. The role of structural aspects of oligonucleotides, 8 to 48 nucleotide strands, on immobilization on gold has been investigated using electrochemistry, phosphorimaging, FT-IR spectroscopy, and neutron reflectivity. The value of incorporating a substrate-specific binding group was confirmed. In the case of binding to gold, the substrate-specific group is a thiol (SH). The packing density was studied as a function of the oligonucleotide length. The packing density is roughly constant for oligonucleotide segments less than 16 nucleotides in length. Longer strands pack on the surface at a density that is inversely proportional to the number of nucleotides in the segment. The data suggests that the conformation of single-stranded DNA transitions from a 'rigid rod' to a 'flexible coil' near this 16 nucleotide segment length.

9:00am **BI-MoM3 Gene Engineering for Biosensor-Biology Interface**, ***M. Aizawa***, *E. Kobatake, Y. Yanagida, T. Haruyama,* Tokyo Institute of Technology, Japan **INVITED**

Biosensor technology has made a remarkable progress in these three decades, which may be characterized by unique integration of immobilization and measurement technologies in the first generation, effective employment of advanced technologies such as microelectronics in the second generation and its own development based on generic technology in the third generation. Up to the second generation, the progress of biosensor technology has been realized by adopting the related technologies. It should be regretful, however, that no biosensor material, for instance, has been designed or synthesized for its own purpose. Much effort has been devoted to adopting a native biomolecule to fit a biosensor. It is not until the third generation that biosensor material is designed for its own purpose on the basis of advanced technology. For the progress of biosensor technology, sensing material design technology should be advanced in harmonization with process technology as well as system technology. As far as sensing material design technology, we should concentrate on designing biosensing materials on the basis of gene engineering for the development of the third generation of biosensors. Gene engineering may fall in three categories of engineering including gene designing of proteins, designing of gene expression process and gene diagnosis. In this paper, both gene designing of proteins and designing of gene expression processes for biosensing are described with focusing on our current achievement.

9:40am **BI-MoM5 Cell-Transistor Coupling**, ***A. Offenhäusser***, Max-Planck Institute for Polymer Research, Germany

In recent years it became conceptually feasible to study small networks of synaptically interactive neurons in vitro. Input (stimulation of a single neuron) and output (recording of electrical signals from individual neurons) control in such a neural network could be achieved by direct coupling of the neural electrical signals to a field-effect transistor (FET) device and metal microelectrodes, opening up the possibility for two-way, nontoxic communication between chips and nerve cells. However, the cell-device coupling is not very well understood and the control of this coupling challenging. An extracellular recording system has been designed for the detection of electrical cell signals.@footnote 1@ A field-effect transistor (FET)@footnote 2@ array has been fabricated which consists of p-channel or n-channel FETs with non-metallized gates. The size of the gates of the 16 FETs are from 16x3 um2 down to 5x1 um2 and are arranged in a 4x4 matrix on 200 and 100 um centers. On the other side extended gate electrode (EGE) arrays were used which are arranged in a 8x8 matrix on 200 and 100 um centers. The gate electrodes are made from gold, titanium and silicides with diameters down to 6 um. The cell-device coupling has been studied using various cell types e.g. neuronal cells, cardiac myocytes, and cells from cell lines. The recorded signals will be discussed on the base of a point contact model where contributions from passive as well as active membrane properties are included. @FootnoteText@ @footnote 1@ C. Sprössler, D. Richter, M. Denyer, A. Offenhäusser, Biosens.& Bioelec. 13, 613-618 (1998). @footnote 2@ A. Offenhäusser, C. Sprössler, M. Matsuzawa, W. Knoll, Biosens.&Bioelec. 12, 819-826 (1997).

10:00am **BI-MoM6 Specific Interactions between Bitoin and Avidin Studied by AFM using the Poisson Statistical Analysis Method**, ***T.P. Beebe***, *Y.-S. Lo, N.D. Huefner, W.S. Chan, B.A. Shiley, F. Stevens,* University of Utah

The interactions between biotin and avidin or streptavidin, a prototypical example of specific biological ligand-receptor systems, were studied by atomic force microscopy (AFM). A unique statistical analysis method which makes use of the properties of the Poisson distribution was applied, and the rupture strength of an individual interaction was obtained from the total pull-off forces measured by the AFM. Tip- and surface-modification chemistries were investigated by X-ray photoelectron spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (TOF-SIMS). The Poisson analysis method has several advantages. It requires no assumptions about the surface energies or contact area between the AFM tip and the substrate, it is not limited by the force resolution of the instrument, and the number of measurements required to extract the individual unbinding force is significantly lower than that required by some other methods. It has been shown that bond rupture strengths are dependent on the rate and duration of force loading applied during the unbinding process. The dynamic nature of bond strengths under external forces has been explored in theory and by several computer simulations, however, only very few experimental studies have been reported. In the present study, we measure the unbinding force of the biotin-streptavidin pairs under various loading rates. The loading rate dependence of bond rupture forces and the comparison of our experimental data to the known thermodynamic properties of the system will be discussed.

10:20am **BI-MoM7 Manipulation of Cellular Interactions with Biomaterials toward aTherapeutic Outcome**, ***M.D. Pierschbacher***, Integra LifeSciences Corporation **INVITED**

A new way of manipulating the manner in which cells interact with biomaterials was made possible with the discovery of arginine-glycine-aspartic acid (RGD) as a major cell recognition signal in the extracellular matrix. This RGD signal has been incorporated into synthetic compounds that can function as antagonist or agonist for a class of cell surface receptors called integrins. In the agonist mode, these compounds can be coupled or bound to wide variety of biomaterials to present a target for the physiological interaction of cells with the surface of these materials through one or more of the integrin types expressed on the cell surface. The agonist activity of these compounds is evident from an in vivo response of faster and more complete tissue integration and a reduction in foreign body response. There are more than 25 different integrin subtypes, and different cell types express a unique subset of these on their surface. More than half of the integrins recognize and bind to a form of the RGD

signal in their natural ligand and can distinguish one form of RGD from another. Specific tissue responses such as vascualization or mineralization can be achieved by immobilizing compounds with the appropriate integrin specificity.

11:00am **BI-MoM9 Investigations into Peptide-tethered Lipid Bilayer Membranes***, **L.C.R. Naumann,*** Max-Planck Institute for Polymer Research, Germany*; E.K. Schmidt,* Institute of Physical and Chemical Research (RIKEN), Japan*; A. Offenhäusser, W. Knoll,* Max-Planck Institute for Polymer Research, Germany

Lipid-functionalised thiopeptides were employed to form peptide-tethered lipid monolayers on gold substrates. Upon fusion of liposomes these monolayers formed tethered lipid bilayer membranes (tBLM's) well designed to incorporate membrane proteins such as H+-ATPase, Na,K-ATPase, cytochrome c oxidase (COX) and the acetylcholine receptor (AChR). Pure peptide-tBLMs and those mixed with a lateral spacer such as thioethanol were investigated with regard to protein incorporation which was followed by surface plasmon resonance spectroscopy (SPS). Electrical properties were assessed simultaneously by impedance spectroscopy (IS). Fluorescence microscopy showed the bilayers to be homogeneous, however, by FRAP measurements fluidity of the membranes was not observed. Fluorescence microscopy was also used to determine the surface concentration of fluorescein labeled COX. Binding assays were performed by SPS of agonists and antagonists of the receptors such as cyochrome c and bungarotoxin. The specificity and sensitivity of the binding assays was increased for primary monoclonal and secondary polyclonal antibodies against COX and the AChR by using an extension of SPS, surface plasmon enhanced fluorescence spectroscopy (SPFS). Proton transport through H+-ATPase from chloroplasts was then investigated with either coupled or non-coupled proton discharge at the gold electrode, depending on the applied potential. In cases where faradaic processes were involved, electrochemical techniques were applied, such as square wave voltammetry and chronoamperometry, where as proton transport across the lipid film was followed by IS. Impedance spectra thus showed characteristic changes as a function of adenosin-triphosphate (ATP) and inhibitor (venturicidin) concentration and/or bias potentials.

11:20am **BI-MoM10 Detection of Immobilized Superparamagnetic Nanosphere Assay Labels using Giant Magnetoresistive Sensors***, **M.C. Tondra,*** Nonvolatile Electronics*; M. Porter,* Iowa State University

Commercially available superparamagnetic nanospheres are commonly used in a wide range of biological applications, particularly in magnetically assisted separations. A new and potentially significant technology involves the use of these particles as labels in nanomagnetic assay applications. This labeling is analogous to that of flourescent beads: the beads are excited and detected with magnetic fields rather than with photons. A major advantage of this technique is that the means for label excitation and detection are easily integrable on a silicon circuit. A preliminary study of this technique demonstrated its basic feasibility, and projected a sensitivity of better than 10@super -12@ Mole.@footnote 1@ This paper presents detailed magnetic and geometric design considerations for this type of assay, and addresses the range of applications over which the technique is appropriate. It is shown that, with proper sensor design and immobilization techniques, integrated magnetoresistive sensors can be used to easily detect the presence or absence of single 1000 nm magnetic microspheres immobilized on the surface of a giant magnetoresistive sensor. Detection of microsphere labels in the 10 to 100 nm range may also be possible if other sensing parameters are compatible. @FootnoteText@ @Footnote 1@ David R. Baselt, Gil U. Lee, Mohan Natesan, Steven W. Metzger, Paul E. Sheehan, and Richard J. Colton, "A Biosensor Based on Magnetoresistance Technology," Biosensors and Bioelectronics, Vol. 13, pp. 731-739 (1998).

11:40am **BI-MoM11 Characterization of S-layer-supported Bilayer Lipid Membranes***, **B. Schuster,*** *D. Pum, U.B. Sleytr,* Universität für Bodenkultur Wien, Austria

Biosensors, based on electrical detection of specific ligand binding become of increasing importance over the last years. Reliable application make great demands on these designed systems like stable membranes with sufficient fluidity and controlled, orientated linkage of sensing molecules to benefit from the various biological interactions. One promising strategy is the application of bacterial-cell-surface-layers (S-layers) as biocompatible and supporting structures for bilayer lipid membranes (BLM's). S-layer are the simplest self-assembly systems that produce crystalline, mono-molecular, isoporous protein lattices with well-defined topographical and physico-chemical properties. Recent studies on S-layer-supported BLM's demonstrated, that the fluidity of the BLM is retained and an enhanced stability is observed as these BLM's reveal a decreased tendency to rupture in the presence of ionophores or pore-forming proteins. Furthermore, S-layer proteins can be recrystallized on solid supports like gold or silicon wafers, and provide a biocompatible, water-containing layer. Attached BLM's exhibit an increased fluidity compared to dextran- or silane-supported BLM's and the stability is significantly enhanced. Thus, S-layer can be used as an alternative to soft polymer cushions and to common tethers to support functional BLM's. Additionally, a second S-layer can be recrystallized on the opposite face of the membrane. This will allow to employ the intrinsic molecular sieving properties and to immobilize a range of biologically functional molecules in a well-defined position and orientation on the S-layer lattice. Thus, BLM's with attached S-layer(s) in combination with new sensor technology might play an important role in the development of biosensors.

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