Friday Morning, November 2, 2001

Biomaterials
Room 102 - Session BI-FrM

Biosensors
Moderator: A.T.A. Jenkins, University of Bath, U.K.

8:20am **BI-FrM1 Planar Membrane Assemblies and Waveguide Optics for Biomolecular Device Applications**, ***S. Saavedra***, University of Arizona **INVITED**

This talk will focus on creation and characterization of supramolecular architectures, composed of polymerized lipid bilayer membranes functionalized with proteins, for use in sensor transduction. Novel optical transduction techniques based on planar waveguide optics will also be discussed.

9:00am **BI-FrM3 TOF-SIMS Analysis of Nucleic Acid Biosensor Chips**, ***H.F. Arlinghaus***, *M. Ostrop, O. Friedrichs, J.C. Feldner,* Universität Münster, Germany

In recent years DNA-chip-technology has been a subject of growing interest for clinical diagnostics as well as for DNA sequencing and forensic. DNA-chips are based on the method of sequencing by hybridization (SBH), where unknown DNA fragments are hybridized to complementary oligodeoxynucleotides (ODN) which are immobilized on a solid surface in an array format. The main variables in SBH are the attachment of the nucleic acid sequences to a solid surface, the conditions for hybridization, and the detection of the hybridized DNA sequences. We have used TOF-SIMS to examine in detail the immobilization process of PNA/ODN and to investigate its ability to detect DNA-fragments hybridized to complementary PNAs. For this purpose we immobilized either silane SA-layers on silicon wafers or DTSP SA-layer on Ag or Au surfaces. PNA and DNA of different concentrations were then bound to these layers. Deprotenated (M-H)@super -@ signal of the different ODN and PNA bases as well as phosphate ions were used to monitor the ODN/PNA concentration. It was found that the immobilization process is strongly dependent on concentration and immobilization time. Under optimized conditions, PNA can be covalently bound to these surfaces. The homogeneity of the immobilized PNA depends on the evaporation time of the PNA solution. After optimizing the immobilization process, complementary and non-complementary DNAs were hybridized to the PNA biosensor chip. Hybridized DNA could be readily identified by detecting the PO@sub 2@@super -@ and PO@sub 3@@super -@ masses. A good discrimination between complementary and non-complementary sequences could be achieved. It can be concluded that TOF-SIMS is a very useful techniques for investigating the complexity of the immobilization and hybridization processes and that SIMS has the potential for providing a rapid method for DNA/RNA sequencing and diagnostics. .

9:20am **BI-FrM4 A "Label-Free" Microchip Array Based Protein-Binding Assay using Surface Plasmon Resonance Imaging**, ***C.E.J. Dentinger***, *D. Martin, P. Wagner,* Zyomyx, Inc.

We demonstrate a microchip array based antibody binding assay that relies on imaging surface plasmon resonance (SPR) for detection of protein binding. Since SPR is sensitive to the index of refraction near a gold surface this assay does not require the binding proteins to be labeled (e.g. fluorescently or with a radio label), making it a "label free" detection technique. For our imaging SPR assay we have developed a method of specifically immobilizing proteins on surfaces that resist non-specific protein adsorption. These surfaces consist of a gold substrate coated with an omega functionalized alkanethiol self-assembled monolayer (asymNHS). The succinimidic ester head group of this monolayer is then reacted with biotin-LC-PEO-amine to make a biotin-coated surface. This surface will bind streptavidin in a manner that leaves some of streptavidin's binding pockets open to immobilize a wide variety of biotinylated proteins. The asymNHS, biotin-LC-PEO-amine, and streptavidin layers are characterized with ellipsometry, and Fourier transform infrared reflection adsorption spectroscopy (FTIRRAS). The "label-free" assay is then performed by immobilizing biotinylated proteins in an array format and monitoring the different antibody-antigen interactions on this array by imaging SPR. We have shown that this surface will bind a second protein only to the areas where the biotinylated proteins are immobilized. We have also demonstrated that with our imaging SPR we have the ability to watch the binding assays in real time.

9:40am **BI-FrM5 Comparative Analysis of Immobilization Strategies for Antibodies and Their Fragments on Biosensor Surfaces**, ***P. Peluso***, *D. Wilson, D. Do, M. Venkatasubbaiah, H. Tran, P. Kernen, K. Witte, B. Heidecker, P. Wagner, S. Nock,* Zyomyx, Inc.

Advancement in the study of proteomics requires the development of microarray biosensors which possess a high degree of sensitivity. Towards this goal, strategies must be optimized to couple biomolecular capture agents to matrices at sufficient densities without compromising their binding properties. Using SPR and radioimmunoassay methods, we have examined the effect of different coupling chemistries on the binding activities of various antibodies and their corresponding Fab forms. Novel methods for attaching antibodies and Fab fragments to surfaces were employed and compared with random coupling procedures. In general, we found oriented attachment to better maintain the binding activity of these molecules. Moreover, oriented-Fab surfaces appear to exhibit the highest molecular density and binding activity. We further extrapolated these findings to performance within a microarray format utilizing a fluorescence-based sandwich assay.

10:00am **BI-FrM6 Electrostatically Assisted SPR: A New Method for DNA Mutation Detection**, ***L.K. Wolf***, *R.J. Heaton, A.W. Peterson,* Boston University*; R.M. Georgiadis,* Boston University, usa

Detection of DNA base-pair mutations is essential for the study and treatment of genetic disorders and disease. Present-day studies use DNA microarrays and fluorescently labeled probes for high-throughput mutation analysis. Recently, we have discriminated between matched and mismatched surface immobilized duplexes by evaluating surface coverage and hybridization kinetics with electrostatically assisted surface plasmon resonance (SPR).@footnote 1@ Here, we show that this method allows label-free in-situ detection of a single A-C base-pair mismatch in a 25-mer oligonucleotide duplex. We also apply electrostatically assisted SPR to other types of mismatches, demonstrating the generality and usefulness of this method to mutation detection. The effects of ionic strength on mismatch discrimination are also investigated. @FootnoteText@ @footnote 1@ R. J. Heaton, A. W. Peterson, and R. M. Georgiadis, Proc. Nat. Acad. Sci. 98, 3701 (2001).

10:20am **BI-FrM7 DNA Assay Sensitivity in the BARC Magnetoresistive Biosensor**, ***M.A. Piani***, NOVA Research, Inc.*; R.J. Colton, M. Miller, J.C. Rife, P.E. Sheehan,* Naval Research Laboratory*; C.R. Tamanaha,* Geo-Centers, Inc.*; L.J. Whitman,* Naval Research Laboratory

The Bead ARray Counter (BARC) biosensor uses DNA microarrays, magnetic microbeads, and giant magnetoresistive (GMR) magnetic field sensors to detect and identify biological molecules.@footnote 1@ The core of the sensor is a small, microfabricated chip containing an array of 64 GMR sensors. Distinct single stranded DNA capture probes are immobilized on each sensor. Complementary DNA in a sample is allowed to hybridize on the chip, and is then labeled with magnetic microbeads that are detected by the GMR sensors. The overall system sensitivity is a convolution of the chemical and GMR sensor sensitivities. The chemical sensitivity is determined by the effectiveness of the hybridization and labeling assay. Our current chemical sensitivity is 10 fM for a 60 minute hybridization of 30 bp oligonucleotides. Our efforts to improve this sensitivity involves two tracks. First, we are working with chemiluminescent assays in order to optimize the immobilization and hybridization steps. Second, we are experimenting with peptide nucleic acid (PNA) capture probes instead of DNA. PNA is a synthetic oligonucleotide analog with a neutral backbone that hybridizes with complementary DNA with a higher affinity then the DNA complement. Furthermore, PNA molecules are resistant to nucleases ("DNAses"), enabling the BARC chip to be reconstituted and reused following treatment with the enzyme. Our initial results with PNA indicate an increase in chemical sensitivity of one to two orders of magnitude. @FootnoteText@ @footnote 1@Edelstein et al., Biosensors & Bioelectronics 14, 805 (2000).

10:40am **BI-FrM8 Study of the Formation and Function of the Cell Membrane Hybrid Layers Containing G-Protein Coupled Receptors at SAM Surfaces**, ***V. Silin***, National Institute of Standards and Technology*; R. Madhusudhana,* Institute of Biochemical Research, India*; J.T. Woodward, K.D. Ridge, A. Plant,* National Institute of Standards and Technology

Cell membrane vesicles can reorganize at an alkanethiol/lipid mixed monolayers or alkanethiol monolayer-coated surface resulting in the formation of cell membrane hybrid (CMH) layers. The ability to form CMH layers at the surface from various cell types expressing G-protein coupled receptors offers a promising method for the rapid screening of potential membrane receptor ligands. In our work CMH layers were formed using

membrane vesicles from monkey kidney COS-1 cells that had been transiently transfected with synthetic human CCR5 chemokine receptors. CMHs were formed on a thiahexa-(ethylene oxide)-octadecane (THEO-C18) thiol surface or on a mixed POPC/THEO-C18 surface. AFM and SPR technique showed that the membrane form continuous layers at the surfaces with a thickness around 4 to 5nm for different samples. CMH surfaces were tested using specific antibodies against CCR5 receptors to check the surface concentration of the receptors at the surfaces and their orientation. The specific binding of the chemokine ligand was detected at this surface over different concentration. The kinetics of the CMH formation, as well as antibody and ligand binding to the surfaces was measured using the SPR technique, which showed extremely good sensitivity for this application. A number of control experiments were carried out with nonspecific antibodies, ligands and membranes to support data for the specificity of the CCR5 receptor response.

11:00am **BI-FrM9 Surface Chemistry for a Membrane-based Biosensor@footnote 1@**, ***C. Cole***, Nova Research, Inc.*; M. Natesan,* Geo-Centers, Inc.*; M. Malito,* Nova Research, Inc.*; R.J. Colton, L.J. Whitman,* Naval Research Laboratory

A multianalyte biosensor has been developed that uses magnetic force to differentiate between specific and nonspecific ligand-receptor/ligand-surface interactions.@footnote 2@ The initial sensor was developed as a 36-well microtitre plate, using surface chemistries developed to modify polystyrene.@footnote 3@ In order to enhance sensitivity, reduce assay time, and increase portability, a second generation sensor is being developed that replaces the polystyrene with an alumina ultrafiltration membrane. Although the use of inorganic supports is not necessarily novel, the application of polymer coatings designed to reject protein adsorption to such a surface is. We have developed methods to introduce desired surface functionalities onto commercial membranes. At the same time, we have addressed the potentially more critical issue of ensuring that our coatings are highly reproducible, thus providing for a biosensor with consistent response. To supplement XPS, semi-quantitative methods have been developed to specifically determine the extent of poly(ethyleneimine) and poly(ethyleneglycol) coverage; resulting lots of derivatized surfaces have been found to be indistinguishable in assay use. The membrane-based biosensor has been used to detect proteins, bacteria, and viral particles in 25 minutes or less, with an enhancement in sensitivity of 2-3 orders of magnitude compared to the initial prototype (e.g., 10@super 3@ pfu/mL MS2 vs. 10@super 5@ pfu/mL on the plate-based sensor). Various arraying technologies are being evaluated, including photolithography with activated biotin, mechanical masking, and arraying through site-specific chemistry. Use of these technologies to produce an arrayed filter for multianalyte detection will be discussed. @FootnoteText@ @footnote 1@ Supported by the Joint Technical Panel for Chemical and Biological Defense @footnote 2@ Lee et al., Anal. Biochem. 287, 261 (2000) @footnote 3@ Metzger et al., J. Vac. Sci. Technol. A 17, 2623 (1999).

11:20am **BI-FrM10 An Electroactive Substrate for Direct Transduction of Biorecognition**, ***J. Yan***, *P. Hampton, G. López,* The University of New Mexico

Direct electrochemical transduction of biorecognition on well-defined and protein-resistant electrode surfaces has recently attracted enormous attention. Herein, we report the design and characterization of self-assembled monolayers (SAMs) presenting protein binding ligands, electroactive probe molecules and protein-resistant oligo(ethylene glycol) moieties that allow direct electrochemical transduction of biorecognition at modified electrodes. The electroactive compound, N-(11-mercaptoundecyl)-N'-carboxymethyl-4,4'-bipyridinium dibromide (1), was selected and synthesized because of the known environmental sensitivity and reversibility of the redox chemistry of the viologen groups (i.e., 4,4'-bipyridyl dications) and potential to derivatize the acid terminus with biological molecules. Mixed SAMs of compound 1 and (1-mercaptoundec-11-yl)tri(ethylene glycol) (2) on gold were prepared by coadsorption from a solution of 1 and 2 (@chi@@sub 2@ = 0.6). N-(5-aminopentyl)biotinamide was subsequently attached to the surface through amide bonds. Cyclic voltammetric, ellipsometric and X-ray photoelectron spectroscopic measurements confirmed the attachment of the biotin ligands and the efficiency of the SAM in preventing nonspecific protein binding. Incubation of the biotin-modified SAM in a phosphate buffered saline (PBS, pH 7.4) containing 30 µg/ml anti-biotin for 2 h resulted in a significant negative shift in the redox potential of viologen moieties and a decrease in the peak currents and the charging currents. Incubation of the biotin-modified SAM in PBS containing biotin-blocked anti-biotin for 2 h, however, showed no change in the redox potential, peak currents and charging currents, indicating that the adsorption was biospecific.

11:40am **BI-FrM11 Crystalline Bacterial S-Layer Proteins: A New Supporting Structure to Separate and Stabilize Lipid Membranes**, ***B. Schuster***, *P.C. Gufler, D. Pum, U.B. Sleytr,* Universitaet fuer Bodenkultur Wien, Austria

A key component in the combination of membrane-associated molecular recognition mechanisms with inorganic surfaces is an ultrathin layer separating the lipid membrane and the solid surface. The demands on this layer are manifold as it should both, stabilize and maintain the fluidity and structural properties of the lipid membrane. This is an essential feature providing an environment for reconstitution and immobilization of membrane proteins under non-denaturing conditions. One promising strategy is the application of bacterial cell surface layers (S-layers) to support (bilayer) lipid membranes (BLMs). S-layer proteins are the simplest self-assembly systems that produce crystalline, nanometer-thick, isoporous lattices with well-defined topographical and physico-chemical properties. S-layer proteins have been recrystallized on solid supports like gold or silicon wafers, or deposited on porous polymer filters. Attached BLMs exhibit an increased fluidity compared to dextran- or silane-supported BLMs and the stability is significantly enhanced. By contrast with the less stable BLM on polymer filters, successful reconstitution of staphylococcal alpha-hemolysin was observed with BLMs separated by an S-layer from the porous support. The unitary conductance of a hemolysin pore was found to be similar reconstituted in S-layer supported BLMs on porous supports and in common folded BLMs. As an alternative to soft polymer cushions, to hybrid or tethered lipid membranes, S-layer supported lipid membranes provide a biomimetic, water-containing environment for transmembrane proteins. Furthermore, composite S-layer/lipid membranes in combination with new sensor technology might play an important role in the development of biosensors.

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