Friday Morning, October 19, 2007

Biomaterial Interfaces

Room: 609 - Session BI-FrM

Microbioanalytical Systems

Moderator: T. Boland, Clemson University

8:00am **BI-FrM1 Tools and Platforms for Single-Cell Biology**, ***B.A. Parviz***, University of Washington **INVITED**

Genome sequence data enable global, high throughput approaches that link genomic differences to the physiological outcomes that ultimately lead to disease. However, the Achilles heel of global approaches is reliance on averaged cell populations. It is becoming increasingly clear that cells are highly heterogeneous in both gene expression and phenotype. Cellular heterogeneity confounds the interpretation of the link between genomics, phenotype, and disease and also the interpretation of response to therapeutic intervention. In fact, heterogeneity underlies most failures of current therapies for cancer. In order to realize the promise of genomics in curing major diseases, it will be necessary to elucidate pathways involved in disease at the single-cell level, to both understand and manipulate the inherent heterogeneity. The goal of the Microscale Life Sciences Center (MLSC) is to develop cutting edge technology for multi-parameter analysis of single cells, and apply this technology to the understanding of biological questions characterized by cellular heterogeneity. The current focus is on disease pathways, and the vision is to address pathways to disease states directly at the individual cell level, at increasing levels of complexity that progressively move to an in vivo understanding of disease. This presentation provides an overview of the activities in the Center and efforts undertaken to this date to develop automated platforms for trapping and manipulation of single cells, micro-scale and nano-scale photonic methods to measure cells parameters such as oxygen consumption, single-cell protein analysis, and nano-scale electronic methods to monitor extracellular molecular traffic

8:40am **BI-FrM3 Reversible Biofunctionalization and Catalytic Activity of a Metabolic Pathway Enzyme in Reusable BioMEMS Devices**, ***X.L. Luo***, *A.T. Lewandowski, G.F. Payne, R. Ghodssi, W.E. Bentley, G.W. Rubloff*, University of Maryland

We report a reversible biofunctionalization strategy for assembling a biocatalytically-active enzyme at localized sites in reusable bioMEMS devices as a first step toward an experimental platform for metabolic engineering applications, e.g. drug discovery. We prefabricate an integrated bioMEMS device and exploit a non-permanent sealing and package design which supports programmable bio-component assembly at selected sites in the completely packaged microfluidic environment. The aminopolysaccharide chitosan is utilized as the interfacial biofunctionalization material for (1) the chemical signal-guided conjugation of chitosan to a Pfs enzyme through its pro-tag, which is genetically engineered at the C-terminal of Pfs and is activated by tyrosinase for the conjugation, and (2) the electric signal-guided electrodeposition of the Pfs-chitosan conjugate to a selective electrode under negative bias in the microfluidic channel. With biofunctionalizaton complete, the Pfs-mediated enzymatic reaction is performed by introducing the substrate S-adenosylhomocysteine (SAH) into the microchannel for conversion into the products S-ribosylhomocysteine (SRH) and adenine. Reaction solutions are collected and analyzed with high-performance-liquid-chromatography (HPLC). Initial studies show that high conversions are achieved at low flow rates, while much lower conversions are achieved at high flow. After reaction, a mild acid wash removes the assembled Pfs-chitosan conjugate and thus removes the catalytic activity. Subsequent biofunctionalization with Pfs-chitosan re-establishes enzymatic activity comparable to the original, illustrating the reversibility of the enzyme assembly and the reusability of our bioMEMS. Storage in PBS buffer at room temperature for 4 days degraded the conversion efficiency only slightly, demonstrating robustness of the assembled enzyme. This work demonstrates (a) the reversible assembly of a Pfs enzyme at a specific electrode address and (b) the efficacy of the metabolic pathway enzyme Pfs in the bioMEMS: Pfs converts SAH to SRH and adenine in one step of a multi-step cell-signaling process (autoinducer-2 production), a quorum sensing phenomenon that determines pathogenicity of bacteria. These results illustrate the capability of the bioMEMS as an efficient and reusable platform in screening potential enzyme inhibitors as antimicrobial drug candidates. This work is supported in part by the Robert W. Deutsch Foundation.

9:00am **BI-FrM4 SPR Microscopy and its Applications to High-Throughput Analyses of Biomolecular Binding Events and their Kinetics**, ***C.T. Campbell***, University of Washington, *G. Kim*, Lumera Corp.

Surface plasmon resonance (SPR) sensing has long been used to study biomolecular binding events and their kinetics in a label-free way. This approach has been extended to SPR microscopy more recently, which is an ideal tool for probing large microarrays of biomolcules for their binding interactions with various partners and the kinetics of such binding. SPR microscopes now make it possible to simultaneously monitor binding kinetics on >1300 spots within a protein microarray with a detection limit of below 1 ng per cm2, or <100 femtograms per spot (< 2 million protein molecules) with a time resolution of 1 s, and spot-to-spot reproducibility within a few percent. The method is label free and uses orders of magnitude less of the precious biomolecules than standard SPR sensing. It also gives the absolute bound amount and binding stoichiometry. Experiments designed to demonstrate that this approach is capable of high-throughput kinetic studies of the binding of small (200-500 Da) ligands onto large protein microarrays will be described.

9:20am **BI-FrM5 Studies of Electroosmotic Mobilities, and Protein Adsorption in Plasma Polymerised Microchannel Surfaces**, *M. Salim, B.J. O'Sullivan, G.J.S. Fowler, G. Mishra, P.C. Wright,* ***S.L. McArthur***, University of Sheffield, UK

Miniaturisation has found its application in many biological, medical and pharmaceutical devices. Its advantages over macroscale systems include fast analysis time and lower sample consumption. These microfluidic devices use of electroosmotic flow (EOF) as the mean of fluid transport, although variations and inconsistencies in EOF may interfere with device performances. Plasma polymerisation can be used to deposit thin films of various chemical functionalities and properties. This study investigates the electroosmotic behaviour and stability of a range of different plasma polymerised and polymer grafted surfaces before and after contact with protein solutions. The results illustrate that plasma polymerised surfaces exhibit high stabilities, enabling EOF runs of more than 3 days without deterioration. Critically, EOF measurments of surfaces after contact with protein solutions illustrate significant changes in EOF with very low levels of protein adsorption. While this is detrimental to the function of the device, it does suggest that these measurements may be a sensitive probe for in channel biofouling.

9:40am **BI-FrM6 Attomolar Toxin Detection with Semi-Homogeneous Assays**, ***S.P. Mulvaney***, *K.M. Myers, P.E. Sheehan, L.J. Whitman*, Naval Research Laboratory

Assays for biomolecular detection are ideally both multiplexed and sensitive, metrics that often require conflicting solutions. For example, microarrays use spatial location for multiplexing, but target capture on the surface will ultimately be diffusion-limited. Alternatively, homogeneous assays offer very efficient target capture, but typically require multiple label types to multiplex. In our semi-homogeneous immunoassays we use microbeads for both target capture and labeling to leverage the advantages of both approaches. The sample is first mixed with secondary antibodies and microbeads that are functionalized against the secondary host. Target molecules are thereby captured onto the beads via the secondary antibodies. The target-loaded beads are then captured onto an antibody microarray, and controlled fluidic forces are applied to preferentially remove nonspecifically bound beads.[1] Finally, the remaining beads are counted to determine the target(s) concentration. Utilizing such assays, we have achieved multiplexed toxin detection, including aM detection of SEB, in <20 min in a variety of complex matrices. Micrometer-scale sensors and beads are optimal for detecting nanoscale biomolecules with practical sensitivity and speed.[2] However, the blending of micro- and nano-scales in such assays leads to some interesting relationships. In diffusion-limited, solid-phase assays, it takes hours-to-days for fM targets to accumulate on a nanosensor, but only seconds-to-minutes on a microsensor. In addition, microscale labels enable fluidic forces to be applied to achieve greater sensitivity and fewer false positives than possible with nanoscale labels. Finally, in contrast to nanoscale labels, individual microbeads can be easily detected. We believe the size mismatch between label and target contributes to the extreme sensitivity of our assays. Each microbead-label confines a very small volume beneath the contact area, thereby creating a high local concentration of target molecules and capture/label antibodies. This confinement greatly increases the effective binding constant, suppressing dissociation and detachment of the label. The relatively large bead size also contributes to the unusual log-linear dose response curves we obtain, that span up to nine orders of magnitude.

[1] Mulvaney, et al., Biosens. Bioelectron., in press.

[2] Sheehan and Whitman, Nano Lett. 5, 803 (2005).

10:00am **BI-FrM7 Affinity Capillary Electrophoresis and Other Separations on a Microfluidic Format**, ***F.A. Gomez***, *A. Brown, M. Piyasena, A. Gaspar, S. Stevens*, California State University, Los Angeles **INVITED**

In this paper, we describe the design and development of novel microfluidic devices (MDs) for electrophoretic and chromatographic separations. One study details our work on through-a-chip partial filling affinity capillary electrophoresis (PFACE) to estimate binding constants of ligands to receptors using as model systems carbonic anhydrase B (CAB, EC 4.2.1.1) and vancomycin from Streptomyces orientalis. Using multilayer soft lithography (MSL), a MD consisting of fluid and control channels is fabricated from poly(dimethylsiloxane) (PDMS) and fitted with an external capillary column. Multiple flow channels allow for manipulation of a zone of ligand and sample containing receptor and non-interacting standards into the MD and subsequently into the capillary column. Upon electrophoresis the sample components migrate into the zone of ligand where equilibrium is established. Changes in migration time of the receptor are used in the analysis to obtain a value for the binding interaction. In a second study we describe the development and study of a disposable and inexpensive MD, fabricated from PDMS incorporating conventional chromatographic reversed-phase silica particles (C18) without the use of frits, permanent physical barriers, tapers or restrictors. A novel external in-line magnetic valve allows for facile packing of the particles. Clamping- and anchor-effects providing the stability and the compactness of the packing were observed. A fiber optics assembly is incorporated onto the chip for detection of species. Food dyes and cephalosporin antibiotics were used to demonstrate the chromatographic applicability of this chip-based chromatographic packing.

10:40am **BI-FrM9 Dual Magnetic-/Temperature-Responsive Nanoparticles for Microfluidic Separations and Assays**, ***J.I. Lai***, *J.M. Hoffman, M. Ebara, A.S. Hoffman, P. Stayton*, University of Washington

Magnetic nanoparticle (mNP) technologies have attracted attention for diagnostic applications because mNPs display potential advantages in their diffusive and superparamagnetic properties. However, their small particle size reduces the magnetic capture efficiency. Therefore, there is a need to design mNPs that can be effectively separated without compromising their diffusive properties. Here we have developed an approach that addresses this challenge in the microfluidic channel setting by using mNPs synthesized from temperature-responsive polymeric micelles. Telechelic poly(N-isopropylacrylamide) (PNIPAAm) polymer chains were synthesized with a dodecyl tail at one end and a reactive carboxylate at the opposite end by the reversible addition fragmentation transfer technique. These PNIPAAm chains self-associate into nanoscale micelles that were used as dimensional confinements to synthesize the mNPs. The Mössbauer spectrum of the resulting mNPs shows two broad quadrupolar doublets with chemical shifts of 0.38 and 0.21 mm/s suggesting that the mNPs contain only Fe^{3+}. The X-ray diffraction spectrum confirms the mNP is γ-Fe_2O_3. The mNPs exhibit a layer of carboxylate-terminated PNIPAAm chains as a corona on the surface. The carboxylate group was used to functionalize the mNPs with biotin which was subsequently bound to streptavidin. The biotinylation increases the mNP size from 7 to 11 nm. The functionalized mNPs can be reversibly aggregated in solution as the temperature is cycled through the PNIPAAm lower critical solution temperature (LCST). The LCST of the mNP is ~ 32 ^{o}C before and after the biotinylation. While the magnetophoretic mobility of the individual mNPs below the LCST is negligible, the aggregates formed above the LCST are large enough to respond to an applied magnetic field. The mNPs can associate with biotinylated targets as individual particles, and then subsequent application of a combined temperature increase and magnetic field can be used to magnetically separate the aggregated particles onto the poly(ethylene glycol)-modified polydimethylsiloxane channel walls of a microfluidic device. When the magnetic field is turned off and the temperature is reversed, the captured aggregates re-disperse into the channel flow stream. The dual magnetic- and temperature-responsive nanoparticles can thus be used as soluble reagents to capture diagnostic targets at a specific channel position with temporal control.

11:00am **BI-FrM10 Rapid Analysis of Species Separation in Multianalyte Integrated Micro/Nano Fluidic Chips using Multivariate Image Analysis**, ***K. Artyushkova***, *M. Bore, A. Evangelista-Lara, G.P. Lopez*, University of New Mexico

This study investigates the potential of multivariate methods (MVA) for identifying electrokinetic separation and estimating velocities of moving species based on analysis of imaging datasets from microfluidic and nanofluidic devices. We have developed an image analysis methodology based on MVA of temporal datasets that is capable of identifying velocities of at least two molecular species from the images where no visible separation of the species has occurred. Among multivariate analysis methods examined are Principal Component Analysis (PCA), Multivariate Curve Resolution (MCR), PARAFAC (parallel factor analysis) and Independent Component Analysis (ICA). These methods allow one to fully exploit the data by analyzing all pixels within images and using the temporal dimension, in contrast with manual methods of visual inspection of images or traditional image processing methods. The methodology has been developed and tested temporal images acquired by fluorescence microscopy capturing separation within nanochannels, microchannels and gel electrophoresis of charged dyes and model protein receptor/ligand systems.

11:20am **BI-FrM11 High Throughput Pharmacological Screening using Cell-Based Biosensors**, ***K. Varghese***, Medical University of South Carolina, University of Central Florida, *P. Molnar, N. Bhargava, M. Das,* University of Central Florida, *M.S. Kindy,* Medical University of South Carolina, *J.J. Hickman*, University of Central Florida, Medical University of South Carolina

In drug development there is a large demand for a system capable of high throughput screening, as well as stable long-term recordings. Cell-based biosensors (CBBs) have the potential to address this demand. CBBs work on the principle of a direct interface between electronics and biological cells, such that the electronics make it possible to quantify a change in the cells' immediate environment. The cell-electronics interface can be modified using different physical properties to vary the adhesive properties of cells. It then becomes possible to promote or inhibit cell adhesion, as well as support preferential attachment of one cell type over another. Cell-based electrophysiology can be broadly divided into two categories - 1) those based on intracellular potentials (e.g. use glass microelectrodes, as in patch clamping) and 2) those based on extracellular potentials. Our research focuses on the latter, wherein extracellular microelectrode arrays are used as a noninvasive and long-term approach for the measurement of biopotentials. The objective of this study is to develop a high throughput CBB where the cell-electronics interface is represented by neurons on Metal Microelectrode Arrays (MEAs). The sensor thus developed should be able to detect acute and chronic effects for a broad range of compounds, at a broad range of concentrations, on neuronal physiology. The inherent properties of this CBB also make it possible to obtain long-term recordings from the neurons. The CBB discussed here consists of a layer of cultured embryonic rat neurons on surface-modified MEAs. The interfaces were modified utilizing self-assembled monolayers and characterized utilizing XPS and contact angle measurements. This system was then used to study the time-dependant effects of Amyloid beta (a causative factor of Alzheimer's Disease) on embryonic rat neurons. Since long-term recordings were relatively easy to obtain, it was possible to observe the effects of amyloid beta, at nanomolar concentrations, over a period of ten hours or more, without cell death. A quantitative description of the effect of this compound on the neuronal system utilizing extracellular recordings will be described. The cells were also characterized by morphology as well as immunocytochemical analysis. Intracellular electrophysiological controls were also performed and will be compared to the results obtained with the solid-state devices.

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