Wednesday Afternoon, October 17, 2007

Nanometer-scale Science and Technology

Room: 615 - Session NS1+BI-WeA

Biological and Molecular Applications of Nanoscale Structures

Moderator: J. Randall, Zyvex Corporation

1:40pm **NS1+BI-WeA1 Multiplexed DNA and Protein Arrays Printed via Dip Pen Nanolithography**, ***N.A. Amro***, *S. Rozhok, T. Renner, J. Fragala, M. Nelson*, NanoInk, Inc.

Miniaturized nucleic acid in the form of nanoarrays will dramatically enhance the sensitivity, and spatial density of chip-based bio-assays. These nanoarrays will improve applications ranging from point-of-care diagnosis to genomic arrays used in basic research by enabling the development of next generation screening technologies that are faster, more sensitive, more reliable, and possibly more cost effective than those presently available in the life sciences market. Nucleic acid bioarrays can be printed using Dip-Pen Nanolithography® (DPN®), a new direct-write spotting technology which generates sub-micron sized features of DNA or protein on solid surfaces. This printing technique offers significant advantages over current microarray printing technologies that suffer from poor spot to spot reproducibility in terms of size, shape, and oligonucleotides density, as well as reproducibility across microarray slides. In this talk we report on recent technical advances in patterning DNA and protein patches with submicron dimensions on glass and metal coated substrates, using new commercially available DPN accessories such as multiple pen arrays and microfluidic pen loading which allows performing multiplexed DNA and protein patterning to fabricate arrays with nanoscale registration and high throughput printing. DNA and protein dot features as small as 200 nm in diameter can be routinely generated. DPN patterning of proteins and oligos, as well as screening for their biological activity, will be shown and discussed in detail.

2:00pm **NS1+BI-WeA2 Cell-Surface Interactions: The Extracellular Matrix as Mechanotransducer**, ***V. Vogel***, ETH Zürich, Switzerland **INVITED**

While cells initially respond to the surface chemistry presented on synthetic materials, they rapidly begin to assemble their own matrix. Cells can thereby sense and transduce a broad range of mechanical forces into distinct sets of biochemical signals that ultimately regulate cellular processes, including adhesion, proliferation, differentiation, and apoptosis. But how is force translated at the molecular level into biochemical signal changes that have the potential to alter cellular behavior? The molecular mechanism of fibronectin's (Fn) extensibility within extracellular matrix fibrils is controversial. Does it originate from the force-induced extension of a compactly folded quaternary structure, or from unfolding of fibronectin modules? Clarification of this issue is central to our understanding whether or not the extracellular matrix can act as a mechanotransducer that converts mechanical forces into well regulated biochemical signal changes. Different fluorescence resonance energy transfer (FRET) labeling schemes were used to differentiate between these two models and we quantified how the conformational changes of fibronectin probed by FRET relate to changes of its overall end-to-end extension. The data clearly demonstrate that cells do indeed mechanically unfold fibronectin. The functional implications of the findings are discussed as well as high resolution structural models derived from steered molecular dynamics (SMD) how force might change the functional states of this and other multidomain proteins.

2:40pm **NS1+BI-WeA4 Carbon Nanotube - Chitosan Sites for Direct Electrical Sensing of Biomolecular Events in BioMEMS**, ***S.L. Buckhout-White***, *S.K. Gupta, M.S. Fuhrer, G.W. Rubloff*, University of Maryland

Direct electrical sensing of biomolecular events is highly desirable in bioMEMS applications such as metabolic engineering platforms or biosensor systems. We are pursuing the development and integration of carbon nanotube (CNT) thin film biosensing devices in the microfluidic systems we employ for recreating metabolic pathways for drug discovery. Since these systems exploit chitosan electrodeposition as a spatiotemporally programmable reaction site for biomolecular binding and enzymatic activity, CNT biofunctionalization using chitosan would enable fabrication of the microfluidics environment with embedded CNT devices so that the devices could serve as active sites for biomolecular events and simultaneously read out those events electrically. We have developed integrated CNT devices, for use as conductance elements and/or FET's, and an easily fabricated, reusable microfluidic system using SU8 channels and a PDMS gasket for non-permanent sealing. In addition, we have electrodeposited chitosan, an amine-rich polysaccharide, onto CNT mats exposed as active sites for biomolecular binding down to 1 μm resolution. Since we have already demonstrated that proteins,[1] nucleic acids, and viruses[2] can be conjugated to localized chitosan sites in bioMEMS and retain their biomolecular functionality, we believe the integrated CNT devices are promising for sensing and controlling biomolecular assembly in multi-site bioMEMS networks that recreate metabolic pathways. This work is supported in part by the Robert W. Deutsch Foundation.

[1]Park, J.J., et al., Chitosan-mediated in situ biomolecule assembly in completely packaged microfluidic devices. Lab on a Chip, 2006. 6(10): p. 1315-1321.
[2]Yi, H.M., et al., Patterned assembly of genetically modified viral nanotemplates via nucleic acid hybridization. Nano Letters, 2005. 5(10): p. 1931-1936.

3:00pm **NS1+BI-WeA5 The Study of FET Flow Control and Separation of Proteins in Nanofluidic Channels**, ***Y.-J. Oh***, University of New Mexico, *D. Bottenus*, Washington State University, *D.N. Petsev*, University of New Mexico, *C.F. Ivory*, Washington State University, *S.R.J. Brueck, G.P. Lopez, S.M. Han*, University of New Mexico

We have studied field-effect-transistor (FET) flow control and separation of proteins in a parallel array of nanochannels (100 nm W ×500 nm D), using scanning laser confocal fluorescence microscopy (SL-CFM) and multiple internal reflection Fourier transform infrared spectroscopy (MIR-FTIRS). For fluidic FET, a DC potential is applied to the gate surrounding an isolated mid-section of the channels under longitudinal electric field along the nanochannels. The gate potential controls the surface charge on SiO_2 channel walls and therefore the ξ-potential. Depending on the polarity and magnitude, the gate potential can accelerate, decelerate, or reverse the flow of proteins. We also analyze a pH shift in the nanochannels according to the surface charge modulation and longitudinal electrical field, using Fluorescein as a pH indicator. Our MIR-FTIR analysis shows that Fluorescein dye molecules are hydrogenated and dehydrogenated in response to the gate bias and subsequent pH shift. We demonstrate that the pH shift affects the FET flow control with SL-CFM analysis. A nanochannel device containing multiple gates is used to improve the controllability of protein flow and to introduce a pH gradient along the channels for isoelectric focusing. A different potential is applied to each gate to differentially control the surface charge on the SiO_2 channel walls and to create a pH gradient along the channels. We also generate a pH gradient along the nanochannels, induced by controlled water electrolysis under longitudinal electrical field. The control and separation of proteins, which have different isoelectric points (pI), by the pH gradient along the nanochannels as a function of gate bias and longitudinal electrical field will be further discussed in this presentation.

4:00pm **NS1+BI-WeA8 Microfabricated Cantilever Arrays for Drug Screening Based on DNA-Drug Intercalation**, ***R. Desikan***, Oak Ridge Associated Universities, *H.M. Meyer III, T.G. Thundat*, Oak Ridge National Laboratory

The ability of a cantilever sensor to generate nanomechanical motion from biomolecular interactions can have wide applications in drug discovery. Microfabricated cantilever arrays offer high sensitivity multiplexed detection in real-time in liquid medium. DNA strands when selectively immobilized on one side of the cantilever surface exhibit surface stress variation during interaction with different chemicals. We have used microfabricated piezoresistive cantilever arrays to demonstrate surface stress variation due to immobilization of ssDNA, hybridization and drug interaction that could form the basis for multiplexed detection of chemicals and drugs intercalating with dsDNA. It is shown that immobilization of ssDNA results in a compressive stress while hybridization results in tensile stress. Intercalation between adjacent base pairs results in stiffening, lengthening and unwinding of the double helix, causing it to expand and lose native conformation. Intercalation of chemicals into immobilized dsDNA produces a characteristic oscillatory response pattern. We have investigated the surface stress pattern due to interaction of chemicals such as ethidium bromide and anti-tumor drugs with dsDNA immobilized on cantilever arrays. The dynamic signature pattern can provide new insight on the kinetics and nanomechanics of DNA-drug intercalation. We have used x-ray photoelectron spectroscopy to carry out elemental analysis in order to confirm the presence of the intercalating agent in the DNA. Microfabricated cantilever arrays find potential applications in drug screening, identification of various toxins, and biological sensing.

4:20pm **NS1+BI-WeA9 Nanofluidic System for Investigating DNA-Force and DNA-Protein Interactions**, ***V.R. Dukkipati***, *S.W. Pang*, University of Michigan

Nanofluidic systems are useful in the studies of single-molecule DNA-protein interactions, nanofluidics, and polymer dynamics. DNA is a flexible polymer with a large aspect ratio of 2 nm in width and length in the order of the micrometers. Due to its unique properties, it is ideally suited for investigations using nanofluidic systems for both biological and mechanical characteristics. We present an integrated nanofluidic system, which is applied to study DNA-protein interactions and DNA-force dynamics. In this system, electrodes are integrated in sealed Si nanochannels by PMMA bonding. Cr/Au electrodes are patterned on a 100 μm thick glass followed by PMMA coating and patterning to expose the electrodes. The 100 μm thick glass allows high resolution imaging of DNA at single molecule level. Si nanochannels varying from 350-500 nm in width are sealed with electrodes using PMMA bonding. A 150 μm wide, 1 μm deep microchannel is connected to each of the fluidic ports as an interface to the nanochannels. The microchannel allows the DNA molecules from the inlet port to have an easy access to the nanochannels. The DNA molecules are pumped into the nanochannel array using both hydrodynamic force and electric field. Using the hydrodynamic force, the DNA molecules near the inlet are pumped by evaporation from the outlet port, which is open to atmosphere. Using 100-300 KHz electric field generated by ac voltage applied across the electrodes integrated in the microchannel, the DNA molecules are driven from the microchannel into the nanochannel array. The linear motion generated by the electric field causes the DNA molecules to move in the nanochannels with velocity varying from 5-40 μm/s. This corresponds to a viscous drag force of 0.04-3.80 pN acting on the DNA molecules, assuming the values of viscosity of the water and the radius of the DNA molecules of 0.5 μm in nanochannels. The nanofluidic system is also used to immobilize and stretch T2-DNA molecules using the protein assisted DNA immobilization (PADI) technique. 350 nm wide and 100 nm deep channels are used to immobilize and stretch λ-DNA molecules up to 12 μm long. Further studies will be conducted on the influence of multiple sources of electric field on DNAs in nanochannels.

4:40pm **NS1+BI-WeA10 Plasma Assisted Production of Chemical Nano-Patterns: Study of the Biosensing Efficiency**, *A. Valsesia*, ***P. Colpo***, *I. Mannelli, P. Lisboa, F. Bretagnol, G. Ceccone, F. Rossi*, European Commission - Joint Research Centre, Italy

The next challenge for the development of analytical devices for biological analysis relies on the ability to design advanced surfaces able to interact properly with the biological world. An increase of several order of magnitude of analysis capacity in biosensing devices together with lower detection limits is envisaged, due to the special interactions between the biomolecules and the nanostructured materials. The most important consequences of the nano-structuring of the bio-interacting surfaces is the immobilization of the biomolecular probes in an active state, limiting the non specific adsorption and the optimisation of their binding site accessibility for the bio-recognition of the target molecules. In our laboratory, we have developed alternative fabrication strategies for the creation of chemically nanostructured surfaces by combining Colloidal Lithography and Electron Beam Lithography with Surface Functionalization Techniques such as Plasma Enhanced Chemical Vapour Deposition (PE-CVD) of bio-functional polymers and Self Assembled Molecular Monolayers (SAM). In particular carboxylic functionalized nano-domes in a PEO-like anti-fouling matrix have been produced. We showed that these chemical nano-patterns are able to immobilize proteins selectively in the carboxylic functional nano-domains, leaving the anti-fouling matrix clear Moreover, we have compared the detection performances between uniformly functionalized surface and chemically nano-patterned surfaces when applied as platforms for antigen/antibody interactions. In particular, homogeneous PAA was compared with the PAA nano-areas in anti-fouling matrix previously described. Nano-patterned surfaces showed a considerable enhancement of the immunoreaction efficiency with respect to the non-structured surfaces, demonstrating the capability of nano-patterns to improve the binding site accessibility of the immobilized biological probes.

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