Wednesday Afternoon, October 17, 2007

Biomaterial Interfaces

Room: 609 - Session BI-WeA

Nucleic Acid Sequencing and Technology

Moderator: L.J. Gamble, University of Washington

1:40pm **BI-WeA1 Surface Initiated Enzymatic Polymerization of DNA**, ***A. Chilkoti***, *D. Chow, S. Zauscher*, Duke University

We demonstrate a technique to synthesize DNA homopolymers on a surface using surface-initiated enzymatic polymerization (SIEP) with terminal deoxynucleotidyl transferase (TdTase), an enzyme that repetitively adds mononucleotides to the 3' end of oligonucleotides. The thickness of the synthesized DNA layer was found to depend on the deoxymononucleotide monomer, in the order of dATP > dTTP >> dGTP - dCTP. In addition, the composition and the surface density of oligonucleotide initiators were also important in controlling the extent of DNA polymerization. Poly(dTTP) synthesized by SIEP was capable of binding to antibodies specific to oligomers of dTTP, indicating that the DNA homolayer is fully functional. TdTase-mediated SIEP can also be used to grow spatially defined three-dimensional DNA structures by soft-lithography and by E-beam nanolithography, and is a new tool for bioinspired fabrication at the micro- and nano-scale.

2:00pm **BI-WeA2 Genome Sequencing with Polony Technology**, ***J.S. Edwards***, University of New Mexico **INVITED**

The resounding success of the Human Genome Project (HGP) clearly illustrates how early investments in developing cost-effective methods of biological data acquisition can have tremendous payoffs for the biomedical community. Over the course of a decade, through refinement, parallelization, and automation of established sequencing technologies, the HGP motivated a 100-fold reduction of sequencing costs, from $10 per finished base to $0.10 per finished base. The relevance and utility of sequencing and sequencing centers in the wake of the HGP has been a subject of recent debate, however, I maintain that the completion of the human genome marks the end-of-the-beginning, rather than the beginning-of-the-end, of the era of DNA sequencing and, more generally, the era of nucleic-acid (NA) technologies. For a wide range of biomedical goals, a strong need is evolving for low-cost NA technology, and I will describe our progress in using polony technology to cheaply and rapidly re-sequence a human genome. The list of realized and potential applications for this type of high-throughput sequencing technology is rich and growing.

2:40pm **BI-WeA4 In Situ Study of Ionic Strength and Probe Coverage Influences on DNA Hybridization using Cyclic Voltammetry**, ***P. Gong***, *K.L. Shepard*, Columbia University, *R. Levicky*, Columbia University and Polytechnic University

Solid-phase hybridization underpins modern microarray and biosensor technologies. While the underlying molecular process, namely sequence-specific recognition between complementary probe and target molecules, is fairly well-understood in bulk solution, this knowledge proves insufficient to adequately understand solid-phase hybridization. Using self-assembled DNA monolayers as a model system for hybridization assays, the influence of ionic strength and probe coverage and their cross-correlation are studied systematically on mm-sized gold electrodes. Electroactive ferrocene and ruthenium compounds were employed to quantify the surface DNA probe and target densities independently. The use of electrochemical labels enables in situ monitoring of the hybridization process as well as quantification of nonspecific versus sequence-specific attachments of targets. Results of these experiments can be summarized in a hybridization "map" as a function of ionic strength and probe coverage. Optimum probe densities that lead to maximum target binding or, alternately, maximum hybridization efficiency under a given set of conditions have been identified. The objective is to obtain better understanding of the physical characteristics of solid-phase hybridization at a more fundamental level and to subsequently use this knowledge to guide DNA microarray and other surface hybridization applications.

3:00pm **BI-WeA5 Hybridization with DNA Probes Bound to Gold by Adenine Nucleotides**, ***A. Opdahl***, *D.F. Shudy*, University of Wisconsin, La Crosse, *L.J. Whitman*, Naval Research Laboratory, *D.Y. Petrovykh*, University of Maryland, College Park, and Naval Research Laboratory

The surface density of immobilized nucleotide probes is a key variable in most applications of DNA-functionalized surfaces because the inter-molecular spacing has a strong impact on subsequent hybridization. In an earlier work, it was demonstrated that probe spacing can be controlled by exploiting the strong and preferential interaction between oligo(dA) and gold.[1] Using a model set of $d(T_m\text{-}A_n)$ oligos, the lateral spacing between DNA molecules was found to be largely determined by the number of nucleotides, n, in the (dA) component; e.g. increasing the number of dA nucleotides in the sequence increased the spacing between probe strands. Here, we use both in situ (SPR) and ex situ (XPS) methods to demonstrate that the surface density of realistic DNA probe sequences can be controlled on gold by incorporating a $d(T_m\text{-}A_n)$ 'tail' in the sequence. We find that surfaces functionalized in this fashion possess many desirable properties, including simplicity in fabrication, highly reproducible hybridization kinetics, and stability over multiple hybridization/melting cycles. The unique feature of our strategy is the relationship between the probe spacing and the length of the dA component in the probe. We find that an even wider range of probe-to-probe spacing can be achieved by co-immobilizing the probe DNA with unmodified oligo(dA), which acts as a lateral spacer. Altering either the length or mole fraction of this spacer systematically changes the probe DNA surface coverage, and thereby systematically modulates the hybridization response. Finally, we will discuss how hybridization with probes immobilized via our approach compares with DNA probes on gold prepared by more conventional strategies.

[1]Opdahl et al., Proc. Nat. Acad. Sci., 104, 9, (2007).

4:00pm **BI-WeA8 Novel Materials and Strategies for DNA Sequencing and Genotyping in Microfluidic Devices**, ***A.E. Barron***, Stanford University, *C.P. Fredlake*, Northwestern University, *J.A. Coyne*, Stanford University, *J.S. Lin, R.J. Meagher*, Northwestern University **INVITED**

High-resolution DNA separations are necessary for electrophoretic DNA sequencing and genotyping, which remains an extremely important workhorse technology even in the present, so-called "post-genomic era". In currently used capillary electrophoresis instruments, polymer networks provide the required molecular sieving of DNA fragments. Electrophoresis in sieving matrices has intrinsic physical limitations in read length, shows reduced performance under high electric fields, and requires capillary loading with viscous polymer solutions. There is an ongoing push toward performing DNA sequencing in miniaturized "lab-on-a-chip" devices, which promise higher throughput and lower cost. We are developing "End-Labeled Free-Solution Electrophoresis" (ELFSE) as way to separate DNA according to size without the need for a sieving matrix. In ELFSE, each DNA molecule in a sample is covalently modified with a unique frictional modifier or "drag-tag" that modifies DNA electrophoretic mobility in a size-dependent fashion. We have designed and synthesized a series of non-natural polypeptide and polypeptoid drag-tags, and conjugated them to DNA for free-solution separations by microchannel electrophoresis. In one approach, artificial genes encoding repetitive polypeptides are constructed by controlled cloning, expressed in E. Coli, and purified. These protein drag-tags have so far have been used to demonstrate 4-color sequencing of ~ 180 bases of DNA by capillary electrophoresis, in the absence of a sieving matrix. The obtainment of longer drag-tags, so that we can get longer reads, is ongoing, and sequencing separations are now being carried out in glass microfluidic chips. The application of hydrophilic polymer wall coatings are a critical element of making this technology work, and this will be discussed in some detail. ELFSE may be the a breakthrough that enables rapid, high-throughput sequencing in integrated microfluidic devices, with all of the accompanying advantages that chips offer.

4:40pm **BI-WeA10 Multivariate Analysis Methods Applied to ToF-SIMS Images of DNA Microarrays**, ***P.-C. Nguyen***, *L.J. Gamble*, University of Washington, *C.-Y. Lee*, 3M Corporate Research Analytical Laboratory, *G.M. Harbers, B.J. Tyler, D.W. Grainger*, University of Utah, *D.G. Castner*, University of Washington

The printing process for preparing DNA microarrays introduces variability in microspots, as observed with fluorescence detection commonly used to analyze these arrays. The nanolitre drops of solution printed onto the microarray surface dries within seconds. During this rapid drying the solution ionic strength and solute concentrations increase dramatically. Interspot variations and non-uniform distribution of probe molecules within spots are major sources of experimental uncertainty in microarray analysis. Various primary ion sources (Bi^+, Bi_3^+, Bi_3^{++}, C_{60}^+, C_{60}^{++} and C_{60}^{+++}) were

used in imaging time-of-flight secondary ion mass spectrometry (ToF-SIMS) to study this non-uniformity. The type of information gained from using different primary ion beams is compared. Principal component analysis (PCA) and maximum autocorrelation factors (MAF) were used to analyze the image results and determine which masses were the main causes of the observed variability. Amine-modified single-stranded DNA was immobilized on commercial slides containing NHS groups. Spots containing 0 to 100% of fluorescent Cy3 labeled DNA were examined. Different percentages of Cy3 label resulted in variations in spot size and shape as well as differences in fluorescence distribution within spots. Imaging ToF-SIMS showed that additives in the print solution (sodium dodecyl sulfate, N-lauroyl sarcosine, salts, etc.) as well as the Cy3 labeled DNA were non-uniformly distributed within the microspots. These non-uniformities were more apparent in images acquired with Bi_3^+ and Bi_3^{++} compared to images acquired with Bi^+. Compared to univariate analysis (i.e., examination of individual masses), both PCA and MAF methods more readily highlighted the distributions of chemical non-uniformities present in the DNA microspots. Results thus far indicate that most detail about types and distribution of chemical species in DNA microspots have been obtained from MAF analysis of the Bi_3^{++} images. However, C_{60}^{++}, C_{60}^{+++} data is providing additional information that is been examined.

5:00pm **BI-WeA11 Quantitative Analysis of Block-oligonucleotide Brushes on Gold**, ***D.Y. Petrovykh***, University of Maryland, College Park, and Naval Research Laboratory, *A. Opdahl*, University of Wisconsin, *L.J. Whitman*, Naval Research Laboratory

DNA brushes with unique properties can be prepared using a new immobilization method that is based on the intrinsic affinity of blocks of adenine nucleotides for gold.[1] Block-oligonucleotides are single-stranded DNA (ssDNA) with sequences that follow, for example, a $d(A_k\text{-}T_m\text{-}N_n)$ pattern: a block of k adenine nucleotides $[d(A_k)]$, followed by a block of m thymine nucleotides $[d(T_m)]$, and a short sequence of n (arbitrary) nucleotides $[d(N_n)]$. Brushes formed by the $d(A_k\text{-}T_m\text{-}N_n)$ block-oligonucleotides are particularly interesting, because they attach to gold via the d(A) blocks and present the d(N) "probe" sequence for hybridization with complementary nucleotides. We are quantitatively characterizing these DNA brushes before, after, and during the hybridization experiments using a combination of x-ray photoelectron spectroscopy (XPS) and surface plasmon resonance (SPR) imaging. Characterizing these systems presents several analytical challenges. First, unambiguous deconvolution of XPS spectral features is not possible for these chemically non-uniform brushes. Second, the low surface density of the d(N) probe sequences, which is required to maintain high hybridization efficiency, results in low XPS intensities. Finally, DNA hybridization is carried out in an aqueous environment, so comprehensive analysis of these experiments requires establishing quantitative correlations between the in situ SPR imaging and ex situ XPS measurements. We addressed these challenges by taking advantage of the high degree of control over the composition and grafting density of ssDNA brushes immobilized via adenine blocks. The resulting sets of samples having systematically varied properties allowed us to observe and quantify by both XPS and SPR the effects of the surface density of ssDNA probes on the resulting hybridization efficiencies. Developing such quantitative methods that combine results of in situ and ex situ analysis is critical for extending well-developed surface analysis techniques to complex biological surfaces and interfaces.

[1]Opdahl, et al., Proc. Natl. Acad. Sci. USA 104, 9 (2007).

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