DNA Topical Conference
Room 311 - Session DN+BI-MoM

DNA Structures and Surfaces

Moderator: D.G. Castner, University of Washington

8:20am **DN+BI-MoM1 Design Rules for the Assembly of DNA Modified Nanoparticles: Influence of Surface Chemistry, Ionic Strength, and a Polycation**, ***K.J. Jeong***, *S. Bhattacharya, D.B. Janes, G.U. Lee,* Purdue University

DNA-modified gold nanoparticles are promising materials for the efficient assembly of hierarchical nanostructures because of their ability to self-assemble specifically. In this study, the affect of the DNA surface immobilization chemistry, ionic strength of the solution, and a polycation on the efficiency of assembly of DNA-modified gold nanoparticles on DNA-modified gold surfaces was quantitatively studied. The hybridization efficiency was studied for DNA gold nanoparticles on DNA surfaces treating with 6-mercapto-1-hexanol (MCH) and it was found to significantly enhance the density of nanoparticles found on the surface. Ionic strength played a complex role on the observed assembly of the particles. In pure water, no assembly was possible because the diffusion of the nanoparticles was prevented by strong electrostatic repulsion. In ionic strengths greater than 0.1M NaCl, electrostatic repulsion was effective only at short ranges so that the nanoparticles can assemble through DNA hybridization. However, at ionic concentrations greater than 0.4M NaCl, a slight decrease in assembly was observed, which is attributed to the partial loss of particles in solution through aggregation. Theoretical analysis of these results based on the surface forces that govern the interactions between DNA-modified gold surfaces - electrostatic, van der Waals, and steric forces - confirms the interpretation above. One important conclusion drawn from the theoretical analysis is that the forces that govern the stability of DNA-gold colloids are not DLVO forces but a mixture of electrostatic and steric interactions. Spermidine, a polycation, was used to irreversibly lock-in the nanoparticles assembled on the surface so that the samples could be rinsed with water and dried. The results found in this article provide new insight into design rules for controlling the efficient assembly of DNA-modified nanoparticles.

8:40am **DN+BI-MoM2 DNA and Protein Microarray Printing on Silicon Nitride Waveguide Surfaces**, ***P. Wu***, Colorado State University, US; *P. Hogrebe,* MSU; *D.W. Grainger,* Colorado State University

All bioanalytical assays using surface-capture of target analytes suffer from non-ideal sensitivity and selectivity. We have recently focused on microarray formats on optical waveguide surfaces to improve assay performance. Sputtered silicon nitride optical waveguide surfaces were silanized and modified with a hetero-bifunctional crosslinker to facilitate thiol-reactive immobilization of contact-printed DNA probe oligonucleotides, streptavidin and murine anti-human interleukin-1b capture agents in microarray formats. X-ray photoelectron spectroscopy (XPS) was used to characterize each reaction sequence on the native silicon oxynitride surface. Thiol-terminated DNA probe oligonucleotides exhibited substantially higher surface printing immobilization and target hybridization efficiencies than non-thiolated DNA probe oligonucleotides: strong fluorescence signals from target DNA hybridization supported successful DNA oligonucleotide probe microarray fabrication and specific capture bioactivity. Analogously printed arrays of thiolated streptavidin and non-thiolated streptavidin did not exhibit noticeable differences in either surface immobilization or analyte capture assay signals. Non-thiolated anti-human interleukin-1@beta@ printed on modified silicon nitride surfaces reactive to thiol chemistry exhibited comparable performance for capturing human interleukin-1@beta@ analyte to commercial amine-reactive microarraying polymer surfaces in sandwich immunoassays, indicating substantial non-specific antibody-surface capture responsible for analyte capture signal.

9:00am **DN+BI-MoM3 DNA: Not Merely the Secret of Life**, ***N.C. Seeman***, New York University **INVITED**

Structural DNA nanotechnology uses the concept of reciprocal exchange between DNA double helices to produce branched DNA motifs, like Holliday junctions, or related structures, such as double crossover (DX), triple crossover (TX), paranemic crossover (PX) and DNA parallelogram motifs. We have worked since the early 1980's to combine DNA motifs, using sticky-ended cohesion, to produce specific structures. From branched junctions, we have constructed DNA stick-polyhedra, whose edges are double helices, and whose vertices are the branch points of DNA branched junctions. These include a cube, a truncated octahedron, and an irregular graph. We have also made topological targets, such as deliberately designed knots and Borromean rings. Recently, we have begun to template the topology of industrial polymers, such as nylon, with DNA-like scaffolds. Nanorobotics are key to the success of nanotechnology. We have used two DX molecules to construct a DNA nanomechanical device by linking them with a segment that can be switched between left-handed Z-DNA with right-handed B-DNA. PX DNA has been used to produce a robust sequence-dependent device that changes states by varied hybridization topology. The sequence-dependent nature of this device means that a variety of them attached to a motif can all be addressed individually. Recently, we have used this device to make a translational machine. A central goal of DNA nanotechnology is the self-assembly of periodic matter. We have constructed micron-sized 2-dimensional DNA arrays from DX, TX and two kinds of parallelogram motifs. We can produce specific designed patterns visible in the AFM from DX and TX molecules. We can change the patterns by changing the components, and by modification after assembly. In addition, we have generated 2D arrays from DNA parallelograms. These arrays contain cavities whose sizes can be tuned by design. Recently, we have used robust triangular motifs to produce honeycomb-shaped arrays.

9:40am **DN+BI-MoM5 Adsorption of DNA-wrapped Carbon Nanotubes on SAM Modified Gold Surfaces**, ***R.A. Zangmeister***, *A.M. Opdahl, M.J. Tarlov,* NIST

We are studying the structure of DNA-wrapped carbon nanotubes (CNTs) on gold surfaces modified with alkanethiol self-assembled monolayers (SAMs). Our goal is to understand how DNA-wrapped CNTs interact with surfaces of varying chemical functionality and to use this knowledge to develop general strategies for controlling the assembly and alignment of DNA-wrapped CNTs on surfaces. Recently, Zheng and coworkers found that single-stranded DNA wraps around CNTs, improving their solubility in aqueous solutions and enabling their separation according to diameter and electronic properties.@footnote 1@ We postulate that the well-defined nature of the DNA-conjugated CNTs will allow their precise assembly and alignment on surfaces. We have examined the adsorption of DNA-wrapped CNTs on hydrophobic, hydrophilic, charged, and spatially patterned alkanethiol SAMs on gold. These samples are characterized using reflection absorption FTIR, scanning electron microscopy, and scanning probe microscopy. We have found that DNA-wrapped CNTs preferentially adsorb to positively charged amine terminated SAMs and to bare gold surfaces versus hydrophobic methyl terminated or negatively charged carboxylic acid terminated SAMs, indicating that electrostatic interactions play a major role in the deposition of these materials. In addition, general strategies for controlling the density and alignment of DNA-wrapped CNTs using alkanethiol SAMs will be discussed. @FootnoteText@ @footnote 1@Zheng, M., et al. Nature Materials 2003, 2, 338-342.

10:00am **DN+BI-MoM6 Surface Characterization of DNA Immobilization on Silane-modified SiO@sub x@**, ***G.M. Harbers***, Colorado State University; *L.J. Gamble, D.G. Castner,* University of Washington; *D.W. Grainger,* Colorado State University

Fundamental studies must better understand and characterize DNA-immobilized surfaces to improve DNA microarray assay performance. DNA probe immobilization and target capture in microarray formats are quantified with standard fluorescence and radiometric assays. However, stable, efficient bulk immobilization methods that faithfully replicate microarray formats, but also permit high-sensitivity surface analysis with XPS and NEXAFS, are desired. While thiol-immobilized DNA probes on gold remain popular, silane coupling layers on oxides are more practical by closely duplicating commercial polymer microarray slides. Yet, silane coupling remains problematic, and common amine-terminated silane chemistry requires heterobifunctional crosslinking to immobilize thiol- or amine-modified oligo-DNA. Both commercial microarray substrates and silanated surfaces contain C, O, and N, confounding DNA surface analysis. Although phosphorus (at ~ 5%) is unique to DNA, at low DNA densities, P is difficult to detect and its XPS binding energies overlap with silicon plasmon peaks, making analysis confusing. Therefore, we compare silane coupling agents and substrates lacking nitrogen to follow unique DNA nitrogen and when possible phosphorus signals. Several amine- and thiol-reactive silanes on silicon oxide substrates were monitored with XPS at each surface coupling stage. In addition to a qualitative assessment of DNA density using XPS with expected N/P ratios of ~3.8, immobilized oligo-DNA densities and hybridization efficiencies were quantified using @super 32@P-DNA radiolabeling. DNA densities scaled with oligo-DNA feed concentrations (5nM-2μM) and target hybridization depended on oligo-DNA densities,

ranging from ~100% at low densities (~10@super 11@ molecules/cm@super 2@) to <10% at high densities (~10@super 13@ molecules/cm@super 2@). NEXAFS, used to determine oligo-DNA probe and target duplex orientation, demonstrated little to no DNA surface orientation under these conditions.

10:20am **DN+BI-MoM7 From Fundamental Properties to Applications of DNA Monolayers**, ***R. Levicky,*** Columbia University **INVITED**

We investigate self-assembled DNA monolayers on metal and dielectric supports. Chains ranging in size from oligonucleotides to gene-sized polymers have been site-specifically attached without detectable side reactions in an end-tethered, "polymer brush" geometry. On metal supports, polythiol-mediated anchoring can be used to provide highly permanent immobilization of the nucleic acid. X-ray photoelectron spectroscopy (XPS), dynamic light scattering, and electrochemical methods have been applied to investigate the charging behavior, counterion partitioning, and organization of DNA monolayers on metal supports, and to evaluate label-free electrochemical approaches for monitoring interfacial hybridization reactions. The interfacial capacitance of end-tethered DNA films has been interpreted within a polyelectrolyte brush model. The observed trends with ionic strength and strand surface coverage generally agree with physical expectations, although as yet not understood increase in capacitance with decrease in ionic strength is observed for densest monolayers. Diagnostic applications are being pursued through development of near-field imaging methods and of active microelectronic substrates that integrate signal detection and processing functionality "on-chip." Near-field measurements offer a label-free technique with a sensitivity comparable to that of fluorescence-based systems currently in widespread use. Microelectronic biochips replace costly macroscopic instrumentation by integration of equivalent function within the solid support, using affordable CMOS microfabrication. Results from validation studies of these emerging technologies and their promise for more portable, simplified, and economical assays will be also described.

11:00am **DN+BI-MoM9 Electric Field Assisted Assembly of 2D and 3D DNA Nanostructures**, ***M.J. Heller,*** University of California, San Diego **INVITED**

Active microelectronic arrays which have been developed for genomic research and DNA diagnostic applications may also have potential for nanofabrication applications. These microarray devices are able to create reconfigurable electric field transport geometries on their surface which allows charged reagent and analyte molecules (DNA, RNA, oligonucleotide probes, amplicons antibodies, proteins, enzymes, nanoparticles and micron sized semiconductor devices) to be moved to or from any of the microscopic test sites on the device surface. These microelectronic array devices have the potential for many nanofabrication applications, including the directed self-assembly of molecular, nanoscale and microscale components into more complex 2D and 3D nanostructures and for creating higher-order mechanisms. This type of electric field assisted self-assembly using active microelectronic arrays is also being investigated as a method to carry out the selective functionalization of nanocomponents with high precision.

11:40am **DN+BI-MoM11 New Approach in Electrochemical DNA Hybridization Detection with Adjacent Impedance Probing**, ***K.S. Ma,*** *H. Zhou, J. Zoval, M. Madou,* University of California, Irvine

Over the past two decades, the rapidly progressing development of electrochemical DNA biosensors have attracted substantial research efforts. Within the large number of electrochemical detection approaches, self-assembled monolayers (SAM) have been shown to be a convenient technique for immobilization of oligonucleotides probes on the gold electrodes. With this type of surface preparation, thiol-labeled ssDNA spontaneously adsorbs onto the gold surface, however, both via thiol-gold linkage (specific) and via non-specific interactions, thus introducing undesirable random orientations of the ssDNA molecules. These random orientations are undesirable because of the resulting non-perfectly-polarized membrane on the electrode. To avoid the problems caused by non-specific adsorption of probes on electrode surface, many methods have been studied intensively. Paleek et al. worked on a new assay method called the "two-surface strategy". In our current study, a unique method was employed for DNA hybridization detection. Using micro-fabrication processes, the two-surface concept was proved to be feasible in the same spatial domain. In this case, the DNA hybridization microspot is made for the bio-recognition event and a bare adjacent conductor electrode is designed for generating the impedance change through insulator deposition. The Electrochemical Impedance Spectroscopy was employed for the measurements. At medium frequency, a noticeable increase of impedance modulus appeared in the Bode plot. For non-complementary measurement, there was no change of impedance modulus. By this biosensor, upon DNA hybridization and subsequent deposition of the enzymatic reaction product, we had found significant improvement on the signal of DNA hybridization detection. Further studies such as lowest detection limitation are required before any practical applications.

DNA Topical Conference
Room 311 - Session DN+BI-MoA

DNA Detection and Sensing
Moderator: R.M. Georgiadis, Boston University

2:00pm **DN+BI-MoA1 Diamond-based Electrical Biosensors for DNA Detection**, ***B. Sun***, *W. Yang, H. Kim, K.-Y. Tse,* University of Wisconsin-Madison; *J.N. Russell, Jr., J.E. Butler,* Naval Research Laboratory; *R.J. Hamers,* University of Wisconsin-Madison

The high stability of diamond makes an attractive material to use for interfacing microelectronics to biological molecules such as DNA. We have investigated the fabrication of diamond-based field-effect transistors in which the surface of the diamond is functionalized with biomolecules of interest, and binding to target molecules in solution produces a change in electrical properties via a field effect. Because the sensitivity depends on the size of the FET, our efforts have been placed on developing small devices, a few microns in size. Measurements in a field-effect transistor geometry can be compared to those made via impedance spectroscopy, as both are sensitive to the impedance of the diamond space-charge region. This talk will discuss the factors controlling the sensitivity and electrical stability of diamond-based electrical biosensing devices, as applied to DNA and other biomolecules.

2:20pm **DN+BI-MoA2 Polymer Replicated Interdigitated Electrode Arrays and Their Application in Multiparameter Molecular Diagnostics**, ***W. Laureyn***, *J. Suls, K. Bonroy,* IMEC, Belgium; *G. Van Reybroeck, P. Jacobs, R. Rossau,* Innogenetics NV, Belgium; *P. Detemple,* IMM GmbH, Germany; *C. Van Hoof,* IMEC, Belgium

The development of DNA-sensor devices attracts substantial research efforts directed to gene analysis, detection of genetic disorders, tissue matching, forensic applications, etc. The electronic transduction of the formation of nucleic acid/DNA complexes using electrodes or semiconductors could provide quantitative information on the DNA-analyte in the sample. InterDigitated Electrode (IDE) arrays show great promise for the label-free detection of nucleic acid hybridization. However, the search for a technology that allows the manufacturing of thin-film IDE arrays on polymers in an easy and affordable fashion, which is compatible with state-of-the-art microfluidics integration technology, has received little attention to date. This paper reports on an innovative method for the affordable manufacturing of polymer based arrays of IDEs with µm dimensions. The reported fabrication process is based on a single and directional metal deposition on an appropriate three-dimensional structure, which is realized in a polymer material using micro-injection molding. The molds are manufactured by electroplating as a reverse copy of a silicon master structure. Using a well-designed combination of so-called microchannels and bumps, a self-contained shadowing effect can be achieved resulting in separated IDE structures after directional metal deposition. In this paper, we report on the full experimental proof-of-principle of the production of such devices and on their further integration in a functional micro-fluidic device. As a demonstrator application, we are currently investigating the label-free detection of post-amplification nucleic acid targets. Using planar 1µm IDEs on silicon, fabricated using deep-UV lithography, we were able to discriminate the hybridization of a 1 nM, 261-nt-long PCR-sample (exon 2 of HLA-DQB) down to a single-base mismatch level. A proof-of-principle on DNA-detection with the molded polymer structures is expected mid 2005.

2:40pm **DN+BI-MoA3 Surface Enzyme Reactions for Enhancing SPR Imaging Measurements of DNA Microarrays**, ***R.M. Corn***, University of California, Irvine **INVITED**

The identification and application of bioaffinity interactions in a large scale array format has become an indispensable tool for modern biological research. Bioaffinity interactions such as DNA-DNA and DNA-protein interactions are now employed in an array format to quickly ascertain the presence of a particular DNA or RNA sequence in a sample, to detect and identify microbial and viral species, and to verify efficacy and function in medical diagnostics. In addition to the detection of DNA, microarrays are can be for the identification of new DNA-protein bioaffinity interactions such as and protein-protein binding. The surface-sensitive optical technique of surface plasmon resonance (SPR) imaging is a powerful "label free" measurement that can be used in an array format for the detection of bioaffinity interactions. SPR imaging detects the presence of a biopolymer on a chemically modified gold surface by the change in the local index of refraction that occurs upon adsorption. This talk will highlight the use of surface DNA enzyme reactions in conjunction with SPR imaging measurements to either provide enhanced biochemical selectivity or to amplify the optical response of the bioaffinity adsorption events. For example, we have recently used the enzyme RNase H to detect DNA adsorption onto RNA microarrays from femtomolar solutions. Further experiments on the reaction of Exo III with double-stranded DNA arrays and the use of Exo I with single-stranded DNA arrays will be employed to detail the kinetics of the surface enzyme reactions, which can be described with a combination of Langmuir and Michaelis-Menten concepts.

3:20pm **DN+BI-MoA5 Electrically Switchable DNA Layers as a Novel Detection Scheme for Bio-Sensing**, ***U. Rant***, *K. Arinaga, E. Pringsheim, M. Grubb,* University of Technology Munich, Germany; *S. Fujita, N. Yokoyama,* Fujitsu Laboratories Ltd., Japan; *M. Tornow, G. Abstreiter,* University of Technology Munich, Germany

Recently, we reported on the electrical manipulation of oligonucleotide layers tethered to gold surfaces.@footnote 1@ By applying alternating AC potentials to the supporting substrate, the orientation of the DNA strands can be dynamically switched with frequencies ranging up to the kHz regime, while the layer conformation is probed in real-time by optical means. Here we present the underlying principles governing the switchability of DNA layers and, in particular, how this novel functionality can be employed for multi-purpose, label-free sensing applications. In principle, the method can be utilized to detect any kind of (bio-) molecules that, upon specifically binding to the grafted probe layer will alter its switching behavior. As a unique feature, the technique allows to monitor the molecular dynamics of the electrically switched layers, which provides novel means to identify and characterize target-probe complexes on surfaces. We demonstrate the versatility and high sensitivity of the technique by the recognition of specific DNA sequences as well as the detection of a model protein system. @FootnoteText@ @footnote 1@ U Rant, K Arinaga, S Fujita, N Yokoyama, G Abstreiter, M Tornow; Nano Letters 4 (2004), 2441-2445

3:40pm **DN+BI-MoA6 DNA Conductance Sensor Platforms Using Nano-scale Break Junctions**, ***A.K. Mahapatro***, *K.J. Jeong, S. Bhattacharya, G.U. Lee, D.B. Janes,* Purdue University

For DNA sensors, a direct electrical readout of DNA selective binding events would enable integration of sensor elements with readout circuits. A possible readout approach involves measurement of the electrical conductivity of DNA strands bridging two narrowly spaced metallic contacts. In this work we describe few-molecule conductance measurements with electromigration-induced break-junctions (EIBJ). The double-stranded(ds) DNA oligionucleotide sequences are GGCGCGCGGGCGGGC-(CH@sub2@)@sub3@-SH-3', GGCGCAAAAACGGGC-(CH@sub2@)@sub3@-SH-3', and HS-(CH@sub2@)@sub6@-CGGAGAGTTGAGCAT-3', and their complements. Lithographically defined Au wires are formed by e-beam evaporation over oxidized silicon substrates silanated with (3-Mercaptopropyl)trimethoxysilane (MPTMS), then subjected to electromigration at room temperature to create nano-gaps. Although the Au wires are initially 2µm wide, gaps with length ~1nm and width ~5nm are observed after breaking, as observed through a field effect scanning electron microscope. ds-DNA was immobilized on the electrodes by assembling the DNA double-strands in an aqueous solution, reacting these solutions with the electrodes in solution, locking the double-helix configuration with a polycation, thorough rinsing with ultrapure water to remove any residual salt, and drying before measurement. The GC-rich, 3' thiol labeled DNA showed approximately 1Gohm resistance, but little conductivity was measured in the AT-rich or 5'thiol labeled DNA. This is consistent with single molecule conduction measurements where enhanced conductivity has been observed in GC-rich DNA. For the GC-rich DNA, higher conductivity is observed for devices immobilized in a higher concentration of salt (NaCl) in the standard phosphate buffer solution, which is attributed to more DNA-molecules immobilized between the electrodes. This study demonstrates that the EIBJ technique can be used to understand the electrical properties in nanometer scale materials such as DNA.

4:00pm **DN+BI-MoA7 Detection of DNA Hybridization on Porous Silicon Surface by Infrared Microspectroscopy**, ***R. Yamaguchi***, *K. Ishibashi, K. Miyamoto, Y. Kimura, M. Niwano,* Tohoku University, Japan

We propose a label-free method of detecting DNA hybridization by using porous silicon (por-Si) in conjugation with infrared (IR) microspectroscopy. In our method, DNA hybridization is detected through an analysis of infrared spectral profiles, and therefore fluorescence tags are not necessary for the hybridization detection. By using a por-Si film as the chip

substrate, we can immobilize a great number of DNA molecules in a small surface area on the chip surface, since por-Si has a quite large effective surface area as compared to a flat Si surface. This may facilitate a high-sensitive detection of DNA hybridization on a small spot. In this study, we have investigated the feasibility of our method by measuring infrared absorption spectra of DNA molecules on por-Si film surfaces. We prepared a por-Si film with straight pores by anodizing a heavy-doped n-type silicon (100) wafer in a mixture of dilute hydrofluoric acid and ethanol. The diameter of straight pores was approximately 25 nm. Single-stranded DNA (target DNA) with 20 bases was immobilized on the por-Si film surface, and then the film surface was exposed to two kinds of probe DNA; one is complementary to the target DNA, and the other is not complementary to the target DNA. We utilized an IR microspectrometer to measure IR absorption spectra of the film before and after exposure to probe DNAs. The working area of the microspectrometer was set at 50Ã-50 Âµm2. For complementary DNA, we observed absorption peaks due to the probe DNA even after the film surface was rinsed with sodium chloride solution. For non-complementary case, on the other hand, no spectral changes were observed. These observations imply that DNA hybridization can be detected using IR microspectroscopy in conjugation with a por-Si based chip.

4:20pm **DN+BI-MoA8 Probing DNA-DNA Interactions between Cytosine (dC) Homo-Oligonucleotides Immobilized on Gold**, ***A.M. Opdahl,*** National Institute of Standards and Technology*; D.Y. Petrovykh,* University of Maryland and Naval Research Laboratory*; H. Kimura-Suda,* National Institute of Standards and Technology*; L.J. Whitman,* Naval Research Laboratory*; M.J. Tarlov,* National Institute of Standards and Technology

We present experimental evidence for strong interactions between cytosine (dC) homo-oligonucleotides immobilized on gold surfaces. It is known that in neutral and acidic pH solutions (dC)-rich oligos can form multistrand structures [e.g. parallel strand, i-motif] via hemiprotonated (C+)(C) base pairing. For surface-immobilized DNA, we find evidence for the existence of these structures by probing the susceptibility of oligo(dC) films to displacement by 1-mercapto-6-hexanol (MCH) as a function of the buffer solution pH and ion composition. The premise of the method is that MCH is less effective at displacing any individual oligos within a film when strong DNA-DNA interactions are present. The structures and coverages of alkanethiol modified (-SH) and unmodified oligo(dC) films were characterized by Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). Specifically, we find that (dC)@sub 25@-SH films are not displaced by MCH in neutral and acidic pH conditions that favor base-base (C+)(C) interactions, but are readily removed by MCH in mildly basic conditions. Both thiol modified and unmodified (dC) films exhibit higher resistance to MCH displacement in the presence of divalent buffer cations. This additional stability is attributed in part to electrostatic crosslinking of the negatively charged phosphate backbones. These results will be discussed along with the possibility of using (C+)(C) base pairing for stabilizing thiol-tethered DNA strands on gold.

4:40pm **DN+BI-MoA9 Quantification of Immobilized and Hybridized Oligonucleotide Surface Density on Commercial Amine-Reactive Microarray Slides using Radiometric Assay, Fluorescence Imaging and X-Ray Photoelectron Spectroscopy**, ***P. Gong,*** *G.M. Harbers, D.W. Grainger,* Colorado State University

In an effort to establish a quantitative understanding of the correlation between immobilized probe DNA density on microarray surfaces and target hybridization efficiency in biological samples, we have characterized amine-derivatized, single-stranded DNA probes attached to amine-reactive commercial microarray slides and its complementary DNA target hybridization using fluorescence imaging, X-ray photoelectron spectroscopy (XPS) and @super 32@P-radiometric assays. Importantly, we have reproduced immobilization efficiencies of DNA probes under microarray formats using high ionic strength and increased DNA concentrations in macroscopic spotted dimensions to permit XPS surface analysis with good reliability and reproducibility. Target hybridization efficiency with complementary DNA was studied on these capture surfaces and shown to exhibit an optimum at intermediate probe densities. The macroscopic model provides a new platform for study of DNA surface chemistry using highly sensitive, quantitative surface analytical techniques. (e.g., XPS, ToF-SIMS) Sensitive @super 32@P-DNA radiometric measurements are now calibrated with more routine XPS DNA signals, facilitating future routine DNA density determinations without the use of hazardous radioactive assay. The objective is to provide new insight into the surface chemistry influences on DNA probe environments that influences the efficiency of target capture form solution in order to improve microarray assay performance.

5:00pm **DN+BI-MoA10 Ion Current Detection of Mono-nucleotide Passing into a Nano-hole Fabricated on Si Wafer**, ***A. Oki,*** *Y. Horiike,* National Institute for Materials Science, Japan

D. Branton et al. demonstrated the electrical sequencing based on measurement of currents generated by passage of single-strand DNA into a 2 nm hole drilled in a cell membrane. But about 5 nm thick membranes do not allow discriminating single molecule of DNA with stacking spacing of 0.34 nm. For the goal, we have studied detection of currents generated from one base flowing into the nano-hole after cutting DNA to each base using a reaction of @lambda@exonuclease. The nano-hole was fabricated as follows: First, the KOH etching fabricated an anisotropic feature on the backside of the wafer through a 44 µm square Si@sub 3@N@sub 4@ mask, thus self-stopping at 31 µm depth. Then, the KOH etching opened an anisotropic feature on the upper side masked by a 5 mm square window, thereby forming a 360 nm diameter hole on the bottom of the upper side. 50 nm thick SiO@sub 2@ film grown on Si and subsequent CVD of 150 nm thick Si@sub 3@N@sub 4@ film filled periphery of the hole. Finally, 4.5 KeV Ar@super +@ ion irradiation removed the Si@sub 3@N@sub 4@ film on the hole position, thus fabricating the nano-size hole by monitoring instant increase of the ion current using a micro-channel plate set under the wafer. The hole size was not observed by SEM. To measure ion currents generated by mono-nucleotide passing the nano-hole, each 500 µM natural mono-nucleotide of dGMP, dCMP, dAMP, TMP was solved in a TE buffer solution, where a pair of a KCl saturated calomel was used as electrodes. Each ion current increased with increasing voltage. At 1.5V, ion currents of dCMP, TMP, dGMP, and dAMP were 45.9, 21.5, 15.5 and 13.8 nA, respectively. If the ion current varied inversely with molecular weight of mono-nucleotide, increases in order of ion currents are understood for dCMP and TMP. However present experimental accuracy must be checked for inverse characteristic of dGMP for dAMP because of small difference between both ion currents.

DNA Topical Conference
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DNA Poster Session

DN-MoP1 DPN-fabricated Au Microarrays on Si(111) Surfaces for the Site-Selective Immobilization of DNA, ***I. Kaoru***, *I. Takahiro, L. SunHyung, S. Nagahiro, T. Osamu,* Nagoya University, Japan

Scanning probe-based lithography allow us to fabricate sub-100 nm structures on surfaces. Dip-pen nanolithography (DPN) is a new promising scanning probe-based tool for fabricating sub-100 nm to many micrometer structures on surfaces, since it is a method for directly depositing metal from an ink-coated atomic force microscope (AFM) tip onto a substrate with a high spatial resolution. In addition, DPN can draw the arbitrary metal patterns on the desired positions without complicated treatments. Thus, it is appropriate to apply DPN to the fabrication of biosensors on Si semiconductor. In order to develop such sensor devices, it is necessary to establish not only a fabrication technique of metal pattern but also an immobilization method of biomolecules on the pattern. In this study, we aim to fabricate Au microarray by DPN and immobilize DNA on the microarray. n-type Si(111) wafer (4-6 Î© cm) were used as substrate. Firstly, the substrate surface was cleaned by a UV/ozone cleaning method. Next, the Si substrate was terminated with hydrogen by wet etching of HF for 15 min. Au microarrays were then directly drawn on hydrogen-terminated silicon substrate using an AFM through DPN. DNA was selectively deposited on the Au microarrays through Au-S bonds. The surface constructed was traced by an AFM and fluorescence-labeled spheres.

DN-MoP3 PNA Microarray Development and DNA Diagnosis with TOF-SIMS, ***H.F. Arlinghaus***, *S. Hellweg,* Westfälische Wilhelms-Universität Münster, Germany*; T. Grehl, E. Niehuis,* ION-TOF GmbH, Germany*; A. Jakob, J. Hoheisel,* Deutsches Krebsforschungszentrum Heidelberg, Germany

TOF-SIMS was used for the detailed examination of the immobilization process of PNA and its hybridization capability to unlabeled complementary DNA fragments. PNA sequences were immobilized onto SiO@sub 2@ and Au surfaces using different linking mechanisms. Unlabeled single-stranded DNA was hybridized to these biosensor chips containing both complementary and non-complementary immobilized PNA sequences. The hybridization of complementary DNA could readily be identified by detecting phosphate-containing molecules from the DNA backbone such as PO@sub 3@@super -@. The achieved discrimination between complementary and non-complementary sequences was very good. Further investigations were conducted regarding the influence of length and type of spacer molecules on the hybridization efficiency. The spacer molecule defines the distance between the PNA molecule and the biosensor chip surface. It was observed that a greater spacer length leads to higher hybridization efficiency. Using different primary ions, secondary ion yield behavior and fragmentation patterns were studied. This included monoatomic ions (Ar@super +@, Xe@super +@, Au@super +@, Bi@super +@) as well as polyatomic and cluster ions (SF@sub 5@@super +@, Au@sub x@@super +@, Bi@sub x@@super +@). It was found that using polyatomic and cluster primary ions resulted in a significantly increased yield of DNA-correlated fragments, resulting in higher signal intensities and better signal-to-noise ratios. It can be concluded that TOF-SIMS is undoubtedly a highly useful technique for identifying unlabeled hybridized DNA on PNA biosensor chips. It may provide a rapid method for DNA diagnostics and is suitable for studying the complexity of the immobilization and hybridization processes.

DN-MoP4 In-situ Observation of DNA Immobilization and Hybridization on Si by Surface Infrared Spectroscopy, ***K. Ishibashi***, *R. Yamaguchi, K. Miyamoto, Y. Kimura, M. Niwano,* Tohoku University, Japan

We have previously proposed a label-free DNA sensing method by the use of infrared absorption spectroscopy (IRAS) in the multiple internal reflection (MIR) geometry, and demonstrated that hybridization of DNA oligomers dissolved in a buffer solution exhibits specific infrared spectral changes.@footnote 1@ In order to apply our method to DNA chips, we need to immobilize single-stranded DNA (ssDNA) oligomers, which are called "probe DNA", on a Si substrate surface. Furthermore, we need to control on the molecular scale the DNA immobilization process on Si to accomplish a precise analysis of DNA on a chip surface. In this study, we have used MIR-IRAS to in-situ investigate the chemical state of a Si substrate surface at each stage of DNA immobilization process, and to in-situ detect hybridization of immobilized DNA oligomers. MIR-IRAS has quite high surface-sensitivity because of the large number of internal reflections, and enables us to in situ monitor the chemical state of a Si surface in aqueous solution. Thiol-modified, 20-based ssDNA oligomers were immobilized on a Si surface that was modified with 3-aminopropyltrimethoxysilane (APTMS) andã??sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SSMCC). On the basis of IRAS data, we confirmed that ssDNA oligomers were immobilized on a Si surface through covalent bonds. The immobilized ssDNA oligomers were subsequently hybridized with complementary ssDNA oligomers in a sodium chloride solution. We observed specific IR spectral structures at the region of 1600 to 1750 cm-1 that are due to DNA hybridization. The present results indicate that MIR-IRAS is a promising technique used for a label-free DNA sensing device. @FootnoteText@ @footnote 1@ Miyamoto K, Ishibashi K, Hiroi K, Kimura Y, Ishii H, Niwano M, Appli Phys. Lett. 86 (2005) 053902-1.

DN-MoP5 Organophosphonate Monolayers as Functionalisation for Silicon Based Biosensing Devices, ***E. Pringsheim***, Walter Schottky Institute, Technical University of Munich, Germany*; M. Dubey,* Princeton University*; K. Buchholz,* Walter Schottky Institute, Technical University of Munich, Germany*; K. Arinaga,* Fujitsu Laboratories Ltd., Japan*; M.D. Carolus, J. Schwartz,* Princeton University*; M. Tornow, G. Abstreiter,* Walter Schottky Institute, Technical University of Munich, Germany

Planar semiconductor sensing devices based on silicon substrates have immense potential for applications such as label-free, fast, and time resolved detection of biomolecule binding events due to their great sensitivity to surface potential changes via the field effect.@footnote 1@ Phosphonate films are particularly suitable as functionalisation for such devices since they are easy-to-apply and provide for stable silicon surface derivatisation due to dense, self-assembled monolayers (SAMs) that bond strongly to the native silicon oxide. These SAMs can be modified with tailored, substituted end groups.@footnote 2@ We have investigated the covalent linkage of thiol-terminated Cy3 labelled DNA-oligonucleotides to functionalized alkylphosphonate layers assembled on silicondioxide by variation of the immobilisation parameters, including concentration of DNA, concentration of sodium chloride and immobilisation time. Hybridization with complementary strands and the coupling of the model protein avidin to biotinylated DNA is detected optically. Concepts for biofunctionalisation of field effect sensor devices via phosphonic acid monolayers will be discussed. @FootnoteText@ @footnote 1@ M. G. Nikolaides et al., ChemPhysChem, vol. 4, 1104-1106 (2003)@footnote 2@ K. S. Midwood et al., Langmuir, vol. 20, 5501-5505 (2004).

DN-MoP6 SPR Imaging Detection of DNA Hybridization/Adsorption with Exonuclease III Enzymatic Reaction, ***H.J. Lee***, *R.M. Corn,* University of California, Irvine

DNA microarrays are emerging as a powerful tool for gene analysis, viral identification, medical diagnostics and many other biological applications. The next generation of DNA microarrays for biosensing will utilize both surface bioaffinity interactions and subsequent surface enzyme reactions on the adsorbed species in order to greatly enhance the specificity and sensitivity of the biosensor. For instance, we have recently demonstrated a novel approach that utilizes the enzyme RNase H in conjunction with RNA microarrays for the direct detection of DNA at femtomolar concentrations with surface plasmon resonance (SPR) imaging. In this presentation, we demonstrate the use of enzyme Exonuclease III, which specifically digests duplex DNA, in conjunction with DNA microarrays for the amplified SPR imaging detection of single stranded target DNA. Kinetic measurements of the surface Exo III reaction as a function of temperature and enzyme concentration were performed using a combination of SPR imaging and SPR fluorescence. Greater insight into the surface enzyme reaction was achieved through analysis using a newly developed kinetics model that couples the contributions of both enzyme adsorption and surface enzyme reaction kinetics.

DN-MoP7 Secondary Structure Effects on DNA Hybridization in Solution and on Surfaces, ***Y. Gao***, *L.K. Wolf, R.M. Georgiadis,* Boston University

The hybridization process in which a DNA probe strand binds to its complementary target, is the basis of many biological assays. For short oligonucleotides in solution, secondary structure effects on duplex formation kinetics are well known. In contrast, these effects are less understood for surface interactions and some recent work concludes that hybridization at surfaces is not affected by secondary structure. In this work, the effect of secondary structure on DNA hybridization is studied

systematically by UV-vis and surface plasmon resonance (SPR) in solution and on the surface, respectively. Hybridization kinetics were measured for thermodynamically equivalent sequences with varying degrees of single-stranded secondary structure. Our results indicate that duplex hybridization in solution is always one or two orders of magnitude faster than hybridization on the surface, depending on the extent of secondary structure. In addition, we find a direct relationship between the number of base pairs involved in secondary structure and the observed association kinetic rate constants in both environments. On the surface, secondary structure also affects hybridization efficiency. DNA sequences lacking secondary structure achieve equivalent hybridization efficiencies regardless of bulk solution ionic strength. However, as ionic strength is reduced, sequences with secondary structure achieve drastically reduced efficiencies. The binding equilibrium is affected because of the competition between duplex formation and single-strand unfolding.

DN-MoP8 Hybridization of Platinum Drug Adducts, ***L. Postelnicu***, *R.M. Georgiadis,* Boston University

Hybridization of platinum drug adducts DNA is generally considered the major pharmacological target of platinum drugs. As such it is of considerable interest to understand the patterns of DNA perturbation. The new antitumor trinuclear platinum compound [{trans-PtCl(NH3)2}2Âµ-trans-Pt(NH3)2{H2N(CH2)6NH2}2]4+ (designated as BBR3464) is a highly charged compound, non-cross resistant with cisplatin in many human tumor xenografts. The enhanced binding of BBR3464 to single stranded DNA and RNA substrates suggests additional pathways for disrupting cellular function different from the traditional cisplatin. Single strand DNA is present during replication, transcription, recombination and repair. Here, we investigate how DNA hybridization is perturbed by the presence of a single platinum drug bound to one of the DNA strands, using BBR3464 and cisplatin DNA adducts. We use surface plasmon resonance (SPR) spectroscopy to monitor both free DNA and DNA adducts hybridization to a surface immobilized complementary DNA strand. We characterize and compare the hybridization rate constants of cisplatin and BBR3464 DNA adducts at different ionic strength. BBR3464 is a large and highly charge structure compared to cisplatin. The results suggest that the charge of the drug is less important in the hybridization event, and the steric hindrances due to different structures may be more important for hybridization efficiency and kinetics.

DN-MoP9 Characterization of dNTP and ssDNA Attached to Diazonium-modified Surfaces, ***D. Barbash***, *J.E. Fulghum, G.P. Lopez,* University of New Mexico

The immobilization of ssDNA to various modified substrates is utilized in wide-ranging applications including DNA microarray technology and molecular wires. Surface and near-surface characterization techniques such as x-ray photoelectron spectroscopy (XPS) and ATR-FTIR have been demonstrated to be useful in the characterization of attached nucleotides (dNTPs). In this work we use dNTP-based systems as a model for more complex systems. The dNTPs can be uniquely identified using either XPS or ATR-FTIR. Orientation of the attached dNTPs was investigated by ARXPS and found to be consistent with the expected attachment chemistry. Principal component analysis (PCA) on the ARXPS data was utilized to both enhance identification of chemical species and to confirm orientation. ARXPS, ATR-FTIR and multivariate analysis methods have been applied to the study of ssDNA attachment to diazotized surfaces. We will discuss the use of these methods, in combination with data on dNTP attachment, for identifying the orientation of attached ssDNA.

DN-MoP10 DNA Mobility through 2-D Entropic Well Lattices with Nanoscale Constrictions, ***E.A. Strychalski***, *H.G. Craighead,* Cornell University

We are examining the interactions of double stranded DNA with novel micro- and nanofluidic geometries in order to gain an understanding of DNA mobilities through these fluidic structures. In particular, we are constructing various two dimensional arrays of wells through which double stranded DNA are electrophoretically driven. Each well serves as an entropic trap, affecting DNA mobility in a way that inhibits the migration of smaller strands relative to longer strands. While this behavior was first described by Han and Craighead in one dimensional entropic arrays,@footnote 1@ our work seeks to establish the effects of extending the entropic array to two dimensions. Our well lattices typically extend 500 microns down the length of a 500 micron wide channel. These channels are at most 50 nanometers deep between micron deep circular wells with well spacings from 5 to 20 microns. The two dimensional structure of our entropic arrays allow the investigation of varied well diameter, lattice structure, lattice constant, and lattice offset angle relative to the direction of the applied electric field. We report on the effectiveness of various entropic well lattices, embedded in nanoscale fluidic channels, to yield DNA separation. @FootnoteText@ @footnote 1@ J. Han, H.G. Craighead, J. Vac. Sci. Technol. A. 17, 2142 (1999).

DNA Topical Conference
Room 311 - Session DN+BI-TuM

DNA Surface Characterization

Moderator: D.W. Grainger, Colorado State University

8:20am **DN+BI-TuM1 First-principles Calculations of DNA Core Level Energies**, ***J.M. Sullivan,*** Northwestern University and Naval Research Laboratory; *D.Y. Petrovykh,* University of Maryland and Naval Research Laboratory; *G.C. Schatz,* Northwestern University; *L.J. Whitman,* Naval Research Laboratory

X-ray photoelectron spectroscopy (XPS) is emerging as a powerful method for characterizing DNA on surfaces.@footnote 1@ The relative positions of core electron binding energies (CBEs) suggest likely binding geometries and strength of chemical bonds, and the peak areas provide a quantitative measure of the coverage. Although CBEs for simple molecules can often be readily assigned to specific adsorption sites and bonding configurations based on historical data, such interpretation for CBEs of DNA is not possible. As a start to developing a comprehensive ab initio understanding of DNA-surface interactions, we are using density functional theory to determine the geometric and electronic configuration of DNA nucleobases, nucleosides, and nucleotides in the generalized gradient approximation. We have initially focused on the nitrogen spectra because these are the easiest to interpret experimentally, arising solely from the nucleobases. Binding energies of core-electrons are evaluated in a variety of standard methodologies including spin-restricted and spin-unrestricted versions of Slater's and generalized transition state methodologies, and a @Delta@KS-type approach in which the CBE is determined directly by the difference between the total energy of the molecule with and without the core hole. We find the theoretical XPS spectra for isolated nucleic components are surprisingly similar to experimental spectra measured on DNA films, suggesting that-although the films are adsorbed on the surface-the underlying electronic structure of the nucleobases is "free-like". @FootnoteText@ @footnote 1@D. Y. Petrovykh, et al., J. Am. Chem. Soc. 125, 5219 (2003); D. Y. Petrovykh, et al., Langmuir 20, 429 (2004).

8:40am **DN+BI-TuM2 Entropic Recoil of Single DNA Molecules from Nanochannels**, ***J.T. Mannion,*** *C.H. Reccius, J.D. Cross, H.G. Craighead,* Cornell University

Entropic recoil of DNA molecules from pillar arrays has previously been reported.@footnote 1@ It has been shown that this effect has the potential to separate molecules according to length.@footnote 2@ A molecule straddling the interface between two microfabricated regions with vastly different volumes of configuration space experiences a force causing it to retract from the region which has less accessible volume. We have tested a device in which DNA molecules recoil from an array of channels as opposed to pillars. Nanochannels (100nm by 100nm) connected to the end of a microchannel (40 microns by 100nm) have been fabricated in a fused silica wafer. This change in geometry eliminates the possibility of herniation, which had previously been observed for molecules recoiling from pillar arrays. Using channels instead of pillars also makes it possible to track a molecule undergoing the recoil process more precisely and to carefully observe the dynamics of this process. As a consequence, we have been able to study the recoil and drag forces in greater detail. In addition we have clearly identified molecules that enter channels in a folded manner, and can observe unfolding events during recoil. @FootnoteText@ @footnote 1@ Turner, S. W. P.; Cabodi, M.; Craighead, H. G. Phys. Rev. Lett. 2002, 88, 128103-1-128103-4.@footnote 2@ Cabodi, M.; Turner, S. W. P.; Craighead, H. G. Anal. Chem. 2002, 74, 5169-5174. .

9:00am **DN+BI-TuM3 Surface Analysis Characterization of DNA-Microarray Chemistry**, ***L.J. Gamble,*** University of Washington **INVITED**

DNA microarrays have received considerable attention in the fields of bio- and nanotechnology due to their importance in the development of biosensing and diagnostic devices. The construction of these surfaces often entails the attachment of presynthesized oligonucleotides onto a derivatized surface. The hybridization efficiency of DNA microarrays and biosensors is determined in part by variables such as the density and orientation of the single stranded DNA oligomers used to build the devices. Surface analysis techniques such as X-ray photoelectron spectroscopy (XPS), near edge X-ray adsorption fine structure, time-of-flight secondary ion mass spectrometry (ToF-SIMS), and surface plasmon resonance (SPR) can aid in producing reliable, quantitative, and reproducible microarray chemistry. Our initial studies have used model surfaces of self assembled monolayers of thiolated DNA on gold to compare surface characterization (XPS and NEXAFS) of the DNA with hybridization efficiency (SPR and radio-labeling). Factors such as the DNA purity, DNA-surface interaction, and the non-fouling capabilities of the background are discussed. Commercial surfaces and surfaces modeled to simulate the commercial substrates are analyzed with surface analysis techniques as well.

9:40am **DN+BI-TuM5 Surface Science of DNA: The Time Has Come**, ***D.Y. Petrovykh,*** University of Maryland and Naval Research Laboratory **INVITED**

The development of practical DNA-based applications in bio- and nanotechnology has uncovered a menagerie of complications, many related to interactions of molecules with surfaces. Fortunately, the tools and methods developed or adapted for analyzing DNA and other biointerfaces are now significantly advanced to make an impact in both basic and applied surface science of DNA. I will focus on the progress in understanding DNA-surface and DNA-DNA interactions resulting from our ex-situ spectroscopic studies by XPS, FTIR, and NEXAFS. Homo-oligonucleotides have emerged as a useful model system for the study of such interactions. A few examples from the surprisingly wide range of phenomena that we have uncovered include polyelectrolyte behavior of weakly-interacting oligo(dT) strands, buffer-induced cross-linking in oligo(dC) films, and singularly high affinity of oligo(dA) for gold. The most practical impact of our analysis of DNA-functionalized surfaces has been quantifying the effects of experimental conditions (concentration, temperature, buffer salt, etc.) on DNA immobilization and hybridization. Improved understanding of nucleobase-dependent interactions should also facilitate the rational design of immobilization chemistries and DNA probe sequences. Ultimately, the challenge is to apply surface science methods to more complex biomolecular systems and processes, such as label-free characterization of DNA hybridization. @FootnoteText@ This work was done in close collaboration with A. Opdahl, H. Kimura-Suda, V. Perez-Dieste, J. M. Sullivan, F. J. Himpsel, M. J. Tarlov, and L. J. Whitman.

10:20am **DN+BI-TuM7 Kinetics and Thermodynamics of DNA Binding at Surfaces and in Solution**, ***R.M. Georgiadis,*** Boston University **INVITED**

This talk will review recent results on DNA oligonucleotide binding at surfaces and in solution. Using surface plasmon resonance spectroscopy for unlabeled oligonucleotide probes and solution oligonucleotide target species, we examine how the kinetics and thermodynamics of apparent binding for DNA/DNA hybridization depends on strand secondary structure, mismatches, and probe attachment chemistry. We show how SPR imaging can be used for quantitative monitoring of the kinetics of drug/DNA binding for an intercalating drug (actinomycin-D) interacting with array surfaces presenting multiple DNA target sequences containing different drug binding sites. SPR imaging is also used for optimization of attachment chemistry and for development of convenient surface coatings that resist non-specific binding of the pentapeptide containing drug. The array results are compared with solution phase measurements and other non-array SPR measurements and discussed in the context of chemical reaction mechanisms.

11:00am **DN+BI-TuM9 Measurement of RNA Density of States, Ionization Energy, Work Function and Charge Injection Barriers to Inorganic Materials**, *M.M. Beerbom,* ***J.P. Magulick,*** *B. Lagel, A.J. Cascio, R. Schlaf,* University of South Florida

In light of recent conductivity measurements on single DNA stands, which yielded a wide range of results from insulating to metallic, it is interesting to investigate the electronic structure of oligonucleotides. In our experiments we directly measured the highest occupied molecular orbital (HOMO) density of states, the ionization energy, the work function and the charge injection barriers to graphite and Au of RNA homopolymers. This was achieved using photoemission spectroscopy (PES) in combination with clean multi-step in-vacuum deposition of RNA thin films using a homebuilt electrospray deposition system. This system allows the deposition of macro-molecular materials with a level of control similar to Knudsen cells directly from solution allowing essentially contamination free fabrication of thin films suitable for PES measurements. Furthermore, multi-step depositions without breaking the vacuum are enabled by this technique. Our presentation will give an introduction to the measurement technique, and introduce the experimental set-up. Data from several experiments on RNA homopolymer interfaces to graphite and Au will be discussed, and conclusions with regard to conductivity measurements made.

11:20am **DN+BI-TuM10 Compression and Free Expansion of Single DNA Molecules in Nanochannels**, ***C.H. Reccius***, *J.T. Mannion, J.D. Cross, H.G. Craighead,* Cornell University

A variety of micromanipulation techniques have been used in the past to study the mechanical properties of DNA.@footnote 1@ Characterization of these properties is important for an understanding of DNA packing into chromatin or bacteriophage heads and also for the verification of theoretical biopolymer models. Extensive experiments have been conducted on stretching DNA in order to study its elasticity.@footnote 2@ In contrast to stretching by an external force, we are investigating the compression and subsequent free expansion of DNA molecules in artificial nanoscale devices. In this work, single lambda DNA multimers were driven through fluidic channels with a diameter of 100 nm by an electric field. Since the radius of gyration of the biomolecules was bigger than the channel diameter, their equilibrium state was no longer a sphere but instead an elongated cylinder.@footnote 3@ Forcing the stretched DNA into a channel constriction led to a compression of the molecule into a tight conformation. When the electric field was turned off, the DNA molecule slowly expanded back to the energetically favorable stretched out conformation. This expansion can be interpreted with the help of a simple polymer model based on self-avoidance effects. @FootnoteText@ @footnote 1@ Strick T. R., Dessinges M-N., Charvin G., Dekker N. H., Allemand J-F., Bensimon D., and Croquette V., Rep. Prog. Phys., 66, 1-45 (2003).@footnote 2@ Smith S. B., Finzi L., and Bustamente C., Science, 258, 1122-1126 (1992).@footnote 3@ Tegenfeldt J. O., Prinz C., Cao H., Chou S., Reisner W. W., Riehn R., Wang Y. M., Cox E. C., Sturm J. C., Silberzan P., and Austin R. H., PNAS, 101, 10979-10983 (2004).

11:40am **DN+BI-TuM11 In-situ Infrared Spectroscopic Study of Protonation of DNA Aggregated at Electrode Surfaces in Aqueous Solution**, ***K. Miyamoto***, *K. Ishibashi, R. Yamaguchi, Y. Kimura, H. Ishii, M. Niwano,* Tohoku University, Japan

Recently we have proposed a method of monitoring hybridization of nucleic acids using infrared absorption spectroscopy (IRAS) in the multiple internal reflection geometry (MIR). The advantages of our method are follows: (1) Fluorescence labeling is not necessary for detection of DNA hybridization (label-free), (2) IRAS provide us with valuable information about conformations of biomolecules, and (3) MIR-IRAS enable us to in-situ monitor biomolecules in aqueous solution. We confirmed that hybridization of DNA can be detected through infrared spectral profiles: Formation of hydrogen bonding between complementary single-stranded (ss-) DNAs induced specific spectral changes in the frequency region of 1600-1750 cm@super -1@.@footnote 1@ Additionally, we demonstrated that when a positive potential is applied to the Si MIR-prism, negatively-charged ss-DNA molecules are aggregated on the prism surface, and that those aggregated ss-DNA molecules show infrared spectral profiles that are quite different from those of isolated DNA molecules floating in the solution. In this study, we have investigated the infrared spectral changes caused by DNA aggregation at an electrode (anode) surface in aqueous solution. By comparing previous IRAS data obtained for the base protonation under acidic condition, we determine that the observe spectral changes are due to protonation of the bases of DNA in the vicinity of the anode (Si MIR-prism) surface where electrochemical reactions generate a large number of protons. Results of ab-initio calculations support our interpretation. @FootnoteText@ @footnote 1@K. Miyamoto, K. Ishibashi, K. Hiroi, Y. Kimura, H. Ishii, M. Niwano, Appl. Phys. Lett. 86, 053902 (2005).

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