DNA Topical Conference
Room Exhibit Hall C&D - Session DN-MoP

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).

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