Nucleic Acids at Surfaces Topical Conference
Room 2014 - Session DN-MoM

Nucleic Acids at Surfaces I

Moderator: D.W. Grainger, University of Utah

8:00am **DN-MoM1 Carbon Nanotube and Thiol Tethered ssDNA Interactions on Gold**, ***R.A. Zangmeister***, *J.E. Maslar, J.G. Kushmerick,* NIST

Exceptional properties of carbon nanotubes (CNTs) such as high tensile strength, high electron mobility, superior thermal properties, and nanometer dimensions have motivated intense efforts to incorporate these materials into useful electronic and sensing devices. The primary challenges of fabricating useful devices from CNTs result from their polydispersity, poor solubility, and the lack of a general method to deposit CNTs with control over position, density, and alignment. The conjugation of CNTs with biological molecules is a potentially promising route to control the deposition of CNTs on device structures. Biomolecules such as DNA, antibodies, and CNT-binding peptide sequences have been used to control the position, and in some cases alignment of modified CNTs on device structures. We are investigating the interaction of single walled CNTs (SWNTs) with gold surfaces modified with end-tethered single stranded DNA (ssDNA) monolayers. We will report preliminary results from reflection absorption FT-IR, Raman, and scanning probe microscopy characterization of this system. We hope to gain adsorption and dispersity information that will enable advancement in measurement methods based on CNT material device structures.

8:20am **DN-MoM2 Identifying Short DNA-Strands with Redox Markers by in situ Scanning Tunnelling Microscopy**, ***M. Grubb,*** Technical University of Denmark, Denmark; *H. Wackerbarth, T. Albrecht, J. Ulstrup,* Technical University of Denmark

In situ scanning tunnelling microscopy (STM) and electrochemistry of short DNA-strands attached to Au(111)-electrodes via a mercaptohexyl linker has shown that the conformation of short single-stranded sequences depend strongly on the sample potential. Without potential control the molecules form a disordered self-assembled monolayer (SAM). Ordered domains form as the sample potential is stepped to more negative values.@footnote 1@ Mercaptohexanol (MCH) was introduced into the monolayer as a lateral spacer molecule to create a mixed DNA/MCH SAM with space for hybridisation. MCH has the same length as the linker unit and forms itself highly ordered SAMs on Au(111)-electrode surfaces, independent of the sample potential. A mixed monolayer was created by first letting a mercaptohexyl-modified single-stranded DNA adsorb to the Au(111)-surface for one hour. This was followed by washing in buffer and then letting MCH adsorb. One hour is not enough to create a complete monolayer consisting only of DNA, and this leaves space for MCH, which also replaces weakly bound DNA. The mixed monolayer showed no order, and it was not possible to distinguish DNA from MCH. This supports previous findings that DNA in disordered conformation has low contrasts in STM. Hexa-ammineruthenium (II)/(III) (RuHex) can, however, be used to identify DNA immobilised on the surface, since it binds specifically to the DNA backbone.@footnote 2@ The bound RuHex provides a high tunnelling contrast (compared to DNA), but the exact mechanism of the charge transfer process is not yet clear. Single-stranded DNA has previously been viewed as a poor charge transfer agent, but this seems to change when RuHex is bound. Studies of the nature of the charge transfer process of the DNA-bound RuHex are in progress. @FootnoteText@ @footnote 1@ H. Wackerbarth, M. Grubb, J. Zhang, A.G. Hansen, and J. Ulstrup, Angew.Chem.Int.Ed., 2004, 43, 198@footnote 2@ A.B. Steel, T.M. Herne and M.J. Tarlov, Anal.Chem. 1998, 70, 4670.

8:40am **DN-MoM3 Hybridization Behavior of DNA/Oligo(ethylene glycol) Functionalized Gold Surfaces in Complex Media**, ***C.-Y. Lee***, *L.J. Gamble,* University of Washington; *D.W. Grainger,* Colorado State University; *D.G. Castner,* University of Washington

Reliable, direct capture of nucleic acid targets from complex media would greatly improve existing capabilities of DNA microarrays and biosensors. This goal remains a challenge for many current nucleic acid detection technologies attempting to produce assay results directly from complex real-world samples. In this study, we investigate the performance of single-strand DNA (ssDNA) adlayers containing short thiolated oligo(ethylene glycol) (OEG) molecules on gold surfaces using DNA target capture from complete bovine serum and from salmon genomic DNA mixtures with varying target concentrations. The attachment chemistry and surface coverage of probe DNA molecules were studied by X-ray photoelectron spectroscopy (XPS). Target DNA hybridization on probe DNA surfaces was monitored by surface plasmon resonance (SPR). SPR measurements of target DNA hybridization from bovine serum and salmon genomic DNA mixtures demonstrated that OEG incorporation into the ssDNA adlayer improved surface resistance to both non-specific protein and DNA adsorption, allowing detection of small DNA target sequences from concentrated, complex biological mixtures. Target hybridization efficiency from serum decreases only slightly (by roughly 20%) as the serum concentration is increased to 50%. In undiluted serum (100%), target hybridization on the probe surface was reduced by approximately 80%. SPR DNA target detection signal in full-length genomic DNA competition is significantly reduced (by 50-80%) compared to DNA target in buffer. The influence of different probe immobilization chemistry and limit of target DNA detection on the ssDNA adlayers from these complex mixtures will be discussed.

9:00am **DN-MoM4 Amino-Silane and Chitosan Coated Silicon Substrates for DNA Extraction**, *P.L. Pasquardini,* ITC-irst, Italy; *F.S. Fiorilli,* Politecnico di Torino, Italy; *F.S. Forti, L. Lunelli, P.C. Pederzolli, V.L. Vanzetti, V.M. Vinante,* ITC-irst, Italy; *G.E. Garrone,* Politecnico di Torino, Italy; ***A.M. Anderle***, ITC-irst, Italy

Lab-on-a-chip technology has been recently attracting much attention due to their increasing applications to biomedical and clinical diagnosis based on genomic analysis. To this purpose, different methods to perform DNA extraction on a chip have been proposed. Recently, amino-coated silicon microchip has been found to show high capacity to bind DNA, due to the occurrence of electrostatic interaction between amine groups and nucleic acids.@footnote 1@ In this contribution a study about the binding efficiency of DNA on amino-modified silicon and about the quantification of recovered DNA is reported. Amine groups were introduced on silicon substrate by coating with an amino-silane molecule and with chitosan. Silicon substrates have been initially covered with silicon oxide grown both thermally and by PECVD. Aminopropyltriethoxysilane (APTES) has been used as organosilane agent to introduce amine groups. Alternatively a natural polymer, chitosan, carrying amines with a lower pK (~6.3)@footnote 2@ was deposited by evaporation from acid solutions. Amino-coated substrates have been characterized by contact angle, AFM, ESCA and fluorescence microscopy. Genomic DNA (extracted from whole blood) and ï¬-phage DNA molecules adhesion was monitored on the surfaces by AFM and fluorescence microscopy and their release in different elution conditions was quantified by fluorescence spectroscopy. @FootnoteText@@footnote 1@T.Nakagawa, T. Tanaka, D. Niwa, T. Osaka, H. Takeyama, T. Matsunaga, Journal of Biotechnology 116 (2005) 105-111 @footnote 2@L. Wu, A. P. Gadre, H. Yi, M. J. Kastantin, G. W. Rubloff, W. E. Bentley, G. F. Payne, and R. Ghodssi, Langmuir 18 (2002) 8620-8625.

9:20am **DN-MoM5 Thymine Homo-oligonucleotides as Model Systems for DNA Surface Science**, ***D.Y. Petrovykh,*** University of Maryland, College Park; *A. Opdahl, H. Kimura-Suda, M.J. Tarlov,* National Institute of Standards and Technology; *L.J. Whitman,* Naval Research Laboratory

Monolayers of thymine homo-oligonucleotides [oligo(dT)] on gold offer two characteristics that make them uniquely appropriate model systems for studying the surface science of DNA. First, strong and distinct spectral signatures simplify characterization by the traditional surface science methods, such as X-ray photoelectron spectroscopy (XPS), Fourier-transform infrared (FTIR) spectroscopy, and near-edge X-ray absorption fine structure spectroscopy (NEXAFS). Second, the interactions between thymine nucleotides (dT) and gold surfaces are rather weak. The immobilization of oligo(dT) then strongly depends on the choice of specific or non-specific linker chemistries, e.g., unmodified, thiolated, or block-oligonucleotides. For all linker chemistries, the structure of the deposited oligo(dT) monolayers is controlled by a wide range of experimental parameters: DNA length and concentration, buffer salt concentration and composition, deposition time, temperature, and post-deposition treatments. The beneficial combination of properties that we found for the oligo(dT) model system enabled us to quantify the effects of immobilization conditions on the surface density and conformation of the deposited DNA monolayers. For example, systematic variation of the salt used in deposition buffers produced significant effects in our experiments. The immobilization efficiency for thiolated oligo(dT) was dramatically and nonlinearly modulated by both the identity and the concentration of the buffer salt cations. These and other observed properties of the oligo(dT) model systems are not strongly nucleobase-specific, therefore the model results can be generalized for controlling the immobilization of DNA oligos with arbitrary sequences.

9:40am **DN-MoM6 Engineering DNA-DNA Surface Interactions***, **P.E. Laibinis,*** Vanderbilt University*; M. Bajaj, I.H. Lee,* Massachusetts Institute of Technology

The selectivity provided by DNA base pairing provides a general strategy for ultimately programming the self-assembly of smaller building block components into larger functional units with specified hierarchical structures. We have developed methods for immobilizing DNA strands to surfaces with controlled structures and at controlled densities for fundamental studies of DNA hybridization. These studies focus on optimizing DNA hybridization events so to maximize interaction energies between species. By systematically varying the surface density of oligonucleotides, we have established the optimal surfaces for selective DNA adsorption, both as free DNA molecules and as species themselves localized on the surface of gold nanoparticles. These optima are different. We have also explored the ability to perform orthogonal supra-particular assembly by generating particles that expose silica and gold surfaces, each of which was selectively functionalized to expose a different DNA sequence on their surfaces. This orthogonal DNA-directed assembly was confirmed by confocal microscopy of the microspheres. These bifacial particles can be selectively functionalized by the adsorption of their respective DNA complements with goals of directing the assembly of complex structures based on these "Janus" particle building blocks. Efforts toward increasing complexity include the development of a trifacial particle that will also be discussed.

10:20am **DN-MoM8 Quantitative XPS Imaging of DNA Microarray Surfaces***, **L.J. Gamble**, C.-Y. Lee,* University of Washington*; G.M. Harbers, D.W. Grainger,* Colorado State University*; D.G. Castner,* University of Washington

Successful development and optimization of DNA-functionalized surfaces for microarray and biosensor applications requires the accurate and quantitative characterization of immobilized DNA chemistry and structure on various substrates. Previous studies showed that X-ray photoelectron spectroscopy (XPS) is well-suited for sensitive characterization of unpatterned DNA surfaces prepared from bulk solution coupling reactions. However, applying techniques such as XPS to microscopic microarray features (ranging from tens to hundreds of micrometers in diameter) remains a challenge. Recent improvements in imaging photoelectron spectroscopy have allowed more detailed studies of micro-patterned surfaces. In this work, XPS imaging and time-of-flight secondary ion mass spectrometry is applied to the study of patterned DNA surfaces relevant to real world microarray applications. Immobilized DNA probe and target surface compositions on two different commercially available microarray polymer slides are compared using microarray and macro-spot format as well as bulk modification. Distribution of DNA molecules on the microarray slides was determined by chemical mapping of unique nucleotides signals (nitrogen and phosphorus). In addition, the relative amount of probe and target DNA molecules on individual micorarray spots was quantified using region of interest scans. Results indicate that microarray printing on commercial microarray slides produces distinct differences in immobilized DNA density in comparison to bulk solution coupling reaction. Differences in spot size and immobilized probe and target DNA densities on the two commercial microarray slides will be discussed.

10:40am **DN-MoM9 DNA Nanostructure Adsorption and Growth on Inorganic Surfaces***, **G. Zuccheri,*** University of Bologna, INSTM and INFM-S3 Center, Italy*; M. Brucale,* University of Bologna, Italy*; B. Samorì,* University of Bologna, and INFM-S3 Center, Italy

The preparation of DNA-based nanostructures is usually accomplished in solution, by the controlled-temperature assembly of a number of oligonucleotides into complex, often multi-modular structures.@footnote 1@ Several techniques are then used to lay the nanostructures on solid surfaces, either to perform further studies (such as with the AFM) or to integrate them on microfabricated devices. The adsorption of nucleic acids on inorganic surfaces can take place with orientational preference as a function of the DNA base sequence, as we have evidenced on mica.@footnote 2@ A fine control of surface adsorption properties could also prove beneficial for the control and tailoring of DNA-based nanostructure growth, as this can be accomplished directly on surfaces. We have evidence that growing DNA nanostructures based on the stable Holliday junction could take place through only some of the possible pathways when performed on the surface, if compared to solution growth. We collected experimental data on a system based on the DNA parallelogram motif introduced by Prof. Seeman where the assembly could be made more efficient to the point that kinetically-trapped unwanted structures could be avoided by forcing the growth to take place while all the components are adsorbed on a surface. As a fringe benefit, the reduction of dimensionality inherent in the surface adsorption enables the assembly to take place at strongly reduced oligonucleotide concentrations if compared to solution assembly. @FootnoteText@@footnote 1@a) Brucale, M.et al. (2006). Trends In Biotechnology 24: 235-243; b) Samorì, B. and Zuccheri, G. (2005). Angew Chem Int Ed 44:1166-1181. @footnote 2@Sampaolese, B. et al. Proc Natl Acad Sci U S A 99(21): 13566-70.

11:20am **DN-MoM11 Interfacial Hybridization Reactions Monitored by Surface Plasmon Optical and Electrochemical Techniques***, **W. Knoll,*** Max Planck Institute for Polymer Research, Germany **INVITED**

This contribution summarizes some of our efforts in designing, fabricating, and characterizing interfacial binding matrices that allow for a sensitive detection of hybridization reactions between surface-attached catcher probe strands and oligonucleotide targets or PCR amplicons from solution. The multilayer architectures that we employ for the in-situ and real-time detection of the association (hybridization) reaction as well as for the dissociation upon rinsing are based on self-assembly strategies using the well-established biotin-streptavindin conjugates. For the optical characterization of the hybridization processes we use two novel surface plasmon optical techniques, i.e., surface plasmon diffraction for label-free detection, and surface plasmon fluorescence spectroscopy with its unmatched sensitivity for monitoring interfacial binding reactions with LOD values in the sub-femtomolar concentration range. In addition, we employ electrochemical methods as complementary techniques to quantify surface reactions. In a first series of experiments we analyze the details of the hybridization between catcher oligonucleotides (typically 15mers with 15 thymines as spacers) and a variety of single stranded targets differing, in particular, in length and the degree of nucleotide mismatch to the catcher sequence. For the detection of PCR amplicons typically 125 -200 bases long we developed a strategy that employs thermal treatment of the samples in order to separate (melt) the double strands followed by quenching the solution at low temperature to a low ionic strength buffer. This way, we prevent rehybridization in solution but rather allow for efficient association of the single sense strands to the sensor surface. This works best for peptide nucleic acids (PNAs) as catcher strands which interact with the DNA independently of the ionic strength.

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