



# Monday Morning, November 13, 2006

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-particle 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 microarray 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. Samori*, 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. 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. 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@ (a) Brucale, M. et al. (2006). Trends In Biotechnology 24: 235-243; (b) Samori, 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-streptavidin 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.

## Nucleic Acids at Surfaces Topical Conference Room 2014 - Session DN-MoA

### Nucleic Acids at Surfaces II

**Moderator:** L.J. Gamble, University of Washington

**2:00pm DN-MoA1 Counter Ion Free DNA Monolayers - Is It Possible?, A. Vilan, D. Peled, S. Guha Ray, S. Daube, H. Cohen, R. Naaman, Weizmann Institute of Science, Israel**

It is a common knowledge that the phosphate groups of DNA are negatively charged and hence balanced by counter ions. However, using XPS we find very small amounts of cations (e.g., Na, Mg) in compact DNA monolayers self-assembled on gold, with no net charge accumulation. This remarkable observation is studied for single and double strand DNA prepared from different buffer solutions. The counter ion concentration is found to systematically decrease under improving monolayer quality, suggesting that the system gains energy by replacing the original counter ions with protons. Discussion of the experimental evidences and possible implications to naturally packed DNA properties will be given. @FootnoteText@ This research was partially supported by the Grand Center.

**2:20pm DN-MoA2 Transport of DNA in Porous Silicon-Based Microarrays by an External Potential Gradient, R. Yamaguchi, K. Ishibashi, K. Miyamoto, Y. Kimura, M. Niwano, Tohoku University, Japan**

We have previously proposed a porous Si (por-Si) DNA microarray in which DNA hybridization can be detected on por-Si layers by infrared (IR) microspectroscopy. Since por-Si has a quite large effective surface area, we can immobilize a great number of DNA molecules in a small surface area on the array surface, which facilitate high-sensitive detection of DNA hybridization on a small area. In our previous work, we showed that DNA hybridization can be monitored through infrared absorption spectral profiles in the region of the base vibration modes. However, the disadvantage of our method is that it takes several hours to put DNA molecules into the por-Si nanopores. In this study, therefore, we have investigated a method of transporting DNA molecules through the nanopores by an externally applied potential. We utilized a tiny solution cell which is separated into two compartments by a plate of por-Si microarray. One of the compartments was filled with single stranded DNA solution, and the other was filled with pure water. We applied electrical potential between the two compartments. As a result, we found that in the presence of an external potential, DNA molecules quickly moved through the por-Si nanopores, and DNA molecules were condensed in the por-Si layers with high efficiency. This suggests that the efficiency of DNA hybridization can be improved by applying an external potential gradient to the DNA microarray.

**2:40pm DN-MoA3 The Charging Response of DNA Brushes, R. Levicky, Polytechnic University; G. Shen, N. Tercero, P. Gong, Columbia University**

Monolayers of polynucleic acids at solid-liquid interfaces are widely encountered in biological research and medical diagnostics, and also provide versatile experimental models for elucidating the interfacial behavior of charged polymers. When a time varying potential is applied across a layer of end-tethered DNA chains, between the underlying solid support and bulk solution, the resultant capacitive charging currents provide information on layer organization as well as means for monitoring binding of analyte species in diagnostic applications. Experimental data on the capacitance of DNA monolayers have been obtained over a range of ionic strengths and chain coverages, and interpreted in terms of monolayer organization using concepts from polymer science and a modified Gouy-Chapman theory of double layer capacitance. Retention of counterions by the monolayers manifests as a decreased susceptibility of the capacitance to the external salt environment. Moreover, the charging response exhibits signatures of structural reorganization whereby the DNA strands stretch or relax with changes in solution ionic strength. A method for non-destructive electrochemical quantification of strand coverage, based on shifts in the reduction potential of redox-active counterions associated with the monolayer, was also developed. The shifts are partially an outcome of electrical work needed to bring additional counterions into the monolayer, against a concentration gradient, in order to preserve monolayer electroneutrality when its counterions are reduced.

**3:00pm DN-MoA4 Electrostatic Forces at the Liquid-Solid Interface: Biochips, B.M. Pettitt, University of Houston**

Interfaces between disparate phases of matter offer large electrostatic field and density gradients changing the local free energy surface and therefore form a challenging set of problems in current chemical physics. Experiments on DNA microarrays have revealed substantial differences in hybridization thermodynamics between DNA free in solution and surface tethered DNA. We have developed a mean field model of the Coulomb effects in 2-D DNA arrays to understand the binding isotherms and thermal denaturation of the double helix. We find that the electrostatic repulsion of the assayed nucleic acid from the array of DNA probes dominates the binding thermodynamics and thus causes a Coulomb induced blockage of the hybridization. The results explain the effects observed in DNA microarrays: dramatic decrease of the hybridization efficiency and the thermal denaturation curve broadening as the probe surface density grows. We demonstrate application of the theory for evaluation and optimization of the sensitivity, specificity and the dynamic range of DNA array devices.

**3:20pm DN-MoA5 DNA Conformation on Surfaces Measured by Fluorescence Self-Interference, B.B. Goldberg, L. Moiseev, C.R. Cantor, A.K. Swan, M.S. Unlu, Boston University**

**INVITED**

The conformation of DNA molecules tethered to the surface of a microarray may significantly affect the efficiency of hybridization. Although a number of methods have been applied to determine the structure of the DNA layer, they are not particularly sensitive to variations in the shape of DNA molecules. Here we describe the application of a novel interferometric technique called Spectral Self-Interference Fluorescence Microscopy (SSFM) to the precise measurement of the average location of a fluorescent label in a DNA layer relative to the surface and thus determine specific information on the conformation of the surface-bound DNA molecules. Using SSFM we have estimated the shape of coiled single-stranded DNA, the average tilt of double-stranded DNA of different lengths, and estimated the amount of hybridization. The data provide important new proofs of concept for the capabilities of novel optical surface analytical methods of the molecular disposition of DNA on surfaces. The determination of DNA conformations on surfaces and hybridization behavior provide information required to move DNA interfacial applications forward and thus impact emerging clinical and biotechnological fields.

**4:00pm DN-MoA7 Investigation of the Electronic Structure of Ribonucleic Acid Homo Polymer Electronic Structure, Ionization Energy and Charge Injection Barriers to Inorganic Materials, J.P. Magulick, Y. Yi, M.M. Beerbom, R. Schlaf, University of South Florida**

We employed electrospray thin film deposition in concert with photoemission spectroscopy (PES) to investigate the electronic structure of ribonucleic acid (RNA) homo polymers, and their interfaces to inorganic materials such as highly oriented pyrolytic graphite (HOPG) and Au. In these measurements RNA homopolymers were injected directly from solution into a high vacuum chamber attached via in-situ transfer to a photoelectron spectroscopy chamber. This enabled the preparation of clean RNA thin films in multi-step deposition sequences starting out at initial sub-monolayer thicknesses to investigate the details of interface formation. The final multi-layer thickness of the films gave insight into the bulk electronic structure. After each deposition step the samples were characterized with PES without breaking the vacuum. Our results indicate distinctly different ionization energies and charge injection barriers between purines and pyrimidines. Purine homopolymers have an ionization energy of about 6-7 eV while pyrimidine homopolymers exceed 8 eV. In order to further investigate these differences, we also investigated the electronic structure of the isolated backbone, and the individual nucleobases using the same experimental protocol. These results gave insight into the.

**4:20pm DN-MoA8 Electrical Manipulation of DNA on Metal Substrates: Electric Interactions, Molecular Dynamics, and Implications of Hydrodynamic Flow, U. Rant, Walter Schottky Institute, Tech. Univ. Munich, Germany; K. Arinaga, Walter Schottky Institute, Germany & Fujitsu Labs Ltd., Japan; C. Hautmann, S. Scherer, E. Pringsheim, Walter Schottky Institute, Tech. Univ. Munich, Germany; S. Fujita, N. Yokoyama, Fujitsu Labs Ltd., Japan; M. Tornow, G. Abstreiter, Walter Schottky Institute, Tech. Univ. Munich, Germany**

We present experimental investigations addressing the response of surface-tethered oligonucleotides to electric fields at the metal/solution interface. By applying AC potentials to the supporting gold substrates, the

# Monday Afternoon, November 13, 2006

DNA layer conformation can be efficiently modulated using driving frequencies ranging up to the kHz regime. Simultaneously, optical energy-transfer methods are employed to monitor the layer structure in-situ and in real-time. We discuss electric interactions between the charged substrate and the DNA and elucidate how the manipulation efficiency is determined by the electrode bias and electrolyte screening effects. Time-resolved measurements reveal intriguing molecular dynamics of nucleic acids in surface-confined fields and are compared to hydrodynamic simulations. In addition, we show the implications of lateral hydrodynamic flow on the DNA layer structure. The presented results are expected to be generally representative for charged polymers exposed to short-ranged electric fields at surfaces, and, moreover, are of significant importance for the design of a novel type of actively controlled biosensors based on switchable DNA layers.

4:40pm **DN-MoA9 Achieving Reliable Microarray Analysis Results Using Competitive Hybridization**, *A. Chagovetz, L. Williams, J. Bishop, S. Blair*, University of Utah

As microarrays migrate from detection of dissimilar analytes to the detection of analytes that have only a single to a few base mismatches, surface hybridization may become competitive. We have simulated competitive hybridization using a finite element analysis model. Our results show that observed dynamic range between the complement and its competitors increases with temperature because of enhanced dissociation of the mismatch target, assuming thermodynamic equilibrium is not reached. Additionally, competitive hybridization can be enhanced by decreasing the immobilized probe concentration on the surface of the substrate. Using the two observations above we propose two different analysis methods for hybridization experiments, one which applies to end-point analysis and the other which is for real-time analysis. The first method uses a labeled multianalyte sample, while the second method is a novel label-less detection mechanism for multianalyte samples. By introducing a label target that is known to be of a lower affinity than the targets to be investigated we can watch the dissociation of the lower affinity species and predict the concentration of the complement. This is verified experimentally using a microscale array of individually controlled heating elements. The heater array was developed at Sandia National Laboratories, using surface micromachining technology.

## Author Index

### Bold page numbers indicate presenter

— A —

Abstreiter, G.: DN-MoA8, 3  
Albrecht, T.: DN-MoM2, 1  
Anderle, A.M.: DN-MoM4, 1  
Arinaga, K.: DN-MoA8, 3  
— B —  
Bajaj, M.: DN-MoM6, 2  
Beerbom, M.M.: DN-MoA7, 3  
Bishop, J.: DN-MoA9, 4  
Blair, S.: DN-MoA9, 4  
Brucale, M.: DN-MoM9, 2  
— C —  
Cantor, C.R.: DN-MoA5, 3  
Castner, D.G.: DN-MoM3, 1; DN-MoM8, 2  
Chagovetz, A.: DN-MoA9, 4  
Cohen, H.: DN-MoA1, 3  
— D —  
Daube, S.: DN-MoA1, 3  
— F —  
Fiorilli, F.S.: DN-MoM4, 1  
Forti, F.S.: DN-MoM4, 1  
Fujita, S.: DN-MoA8, 3  
— G —  
Gamble, L.J.: DN-MoM3, 1; DN-MoM8, 2  
Garrone, G.E.: DN-MoM4, 1  
Goldberg, B.B.: DN-MoA5, 3  
Gong, P.: DN-MoA3, 3  
Grainger, D.W.: DN-MoM3, 1; DN-MoM8, 2  
Grubb, M.: DN-MoM2, 1  
Guha Ray, S.: DN-MoA1, 3  
— H —  
Harbers, G.M.: DN-MoM8, 2

Hautmann, C.: DN-MoA8, 3  
— I —  
Ishibashi, K.: DN-MoA2, 3  
— K —  
Kimura, Y.: DN-MoA2, 3  
Kimura-Suda, H.: DN-MoM5, 1  
Knoll, W.: DN-MoM11, 2  
Kushmerick, J.G.: DN-MoM1, 1  
— L —  
Laibinis, P.E.: DN-MoM6, 2  
Lee, C.-Y.: DN-MoM3, 1; DN-MoM8, 2  
Lee, I.H.: DN-MoM6, 2  
Levicky, R.: DN-MoA3, 3  
Lunelli, L.: DN-MoM4, 1  
— M —  
Magulick, J.P.: DN-MoA7, 3  
Maslar, J.E.: DN-MoM1, 1  
Miyamoto, K.: DN-MoA2, 3  
Moiseev, L.: DN-MoA5, 3  
— N —  
Naaman, R.: DN-MoA1, 3  
Niwano, M.: DN-MoA2, 3  
— O —  
Opdahl, A.: DN-MoM5, 1  
— P —  
Pasquardini, P.L.: DN-MoM4, 1  
Pederzoli, P.C.: DN-MoM4, 1  
Peled, D.: DN-MoA1, 3  
Petrovykh, D.Y.: DN-MoM5, 1  
Pettitt, B.M.: DN-MoA4, 3  
Pringsheim, E.: DN-MoA8, 3

— R —

Rant, U.: DN-MoA8, 3  
— S —  
Samori, B.: DN-MoM9, 2  
Scherer, S.: DN-MoA8, 3  
Schlaf, R.: DN-MoA7, 3  
Shen, G.: DN-MoA3, 3  
Swan, A.K.: DN-MoA5, 3  
— T —  
Tarlov, M.J.: DN-MoM5, 1  
Tercero, N.: DN-MoA3, 3  
Tornow, M.: DN-MoA8, 3  
— U —  
Ulstrup, J.: DN-MoM2, 1  
Unlu, M.S.: DN-MoA5, 3  
— V —  
Vanzetti, V.L.: DN-MoM4, 1  
Vilan, A.: DN-MoA1, 3  
Vinante, V.M.: DN-MoM4, 1  
— W —  
Wackerbarth, H.: DN-MoM2, 1  
Whitman, L.J.: DN-MoM5, 1  
Williams, L.: DN-MoA9, 4  
— Y —  
Yamaguchi, R.: DN-MoA2, 3  
Yi, Y.: DN-MoA7, 3  
Yokoyama, N.: DN-MoA8, 3  
— Z —  
Zangmeister, R.A.: DN-MoM1, 1  
Zuccheri, G.: DN-MoM9, 2