Biomaterial Interfaces
Room: Hall D - Session BI-ThP

Biomaterial Interfaces Poster Session with Focus on Engineered Bio-Interfaces and Sensors

BI-ThP2 High-Sensitivity Surface Enhanced Raman Scattering of Sub-Picomole Level of Adenine and Thymine Species at Au/Ag Nanoparticle Modified Silicon Nanotip Arrays, ***H.C. Lo***, National Chiao-Tung University, Taiwan, *H.I. Hsiung*, National Taiwan University, *Sv Chattopadhyay*, National Yang-Ming University, Taiwan, *C.F. Chen*, Ming-Dao University, Taiwan, *J. Leu*, National Chiao-Tung University, Taiwan, *K.H. Chen*, Academia Sinica, Taiwan, *L.C. Chen*, National Taiwan University

Optical sensing of adenine and thymine nucleic acid species have been achieved at the femtomolar level using self assembled gold and silver nanoparticles coated silicon nanotips (SiNTs) arrays. The use of sub-10 nm metal particulates with optimum density and inter-particle distance ensures such high levels of sensitivity in surface enhanced Raman scattering experiments. In this work wafer-scale silicon nanotip arrays were fabricated using a patented self masked dry etching technique to provide an excellent platform for the metal self assembly. This structure consists of the SiNTs with apex and bottom diameter of ~ 1 nm and ~ 100 nm, respectively, length of ~ 1000 nm and density of $10^{11}/cm^2$. The high density of gold and silver nanoparticles and short inter-particle distance enabled the bio-immobilization and amplification of the Raman signals of adsorbed molecules, allowing identification of minute amount of the adsorbed molecules with chemical specificity. The high sensitivity of surface enhanced Raman scattering can be maintained over a considerable period of time. The vibrational Raman signals of immobilized species can be detected even after four months of conservation. The straightforward, binder-less, stable and room temperature bio-molecular detection underlines the effectiveness of surface enhanced Raman scattering vis-á-vis fluorescence.

BI-ThP3 Characterization of Functionalized Layers on Silica Surfaces for DNA Attachment, ***R.A. Shircliff***, *J.F. Fennell*, Colorado School of Mines, *I.T. Martin*, *P. Stradins*, National Renewable Energy Laboratory, *S.G. Boyes*, Colorado School of Mines, *M.L. Ghirardi*, National Renewable Energy Laboratory, *S.W. Cowley*, Colorado School of Mines, *H.M. Branz*, National Renewable Energy Laboratory

The morphology and chemistry of functionalized silica surfaces have been characterized to understand key factors to surface uniformity and reproducibility of DNA immobilization and hybridization. Deposited 3-aminopropyltriethoxysilane (APTES) and 3-aminopropyldimethylethoxysilane (APDMES) layers were characterized by x-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), spectroscopic ellipsometry, thermogravimetric analysis, contact angle and DNA bioassays. DNA bioassays included fluorescence-based analysis and ^{32}P-radiometric assays of DNA surface density. Angle-resolved XPS confirmed attachment of the sulfo-EMCS heterobifunctional crosslinker to amine-terminated layers deposited from both APTES and APDMES. High levels of immobilization of thiolated single-strand DNA to APTES-modified surfaces were observed by fluorescence from fluorescein dye attached to the DNA. Surprisingly, there was no detectable attachment of thiolated DNA to surfaces modified with monolayer films from APDMES. AFM of the APTES film revealed up to micron-scale island formations, which were likely caused by polymerization in the solution phase or on the surface. The APTES films also had significant variations of morphology under nominally identical deposition conditions, which may correlate with irreproducibility in DNA attachment. In contrast, the APDMES films had sub-nanometer surface roughness. Deposition of APTES, commonly used in DNA microarrays, showed high immobilization efficiency but lacked good reproducibility. APDMES films, which can only form a monolayer, showed reproducible monolayer films but lacked measurable DNA attachment. As an alternative to silane films, preliminary results will be reported on poly(ethylene glycol)-based films in order to improve reproducibility of DNA immobilization. We gratefully acknowledge the NREL Laboratory Directed Research and Development program for project funding. One of us (JFF) was supported by the U.S. Army.

BI-ThP4 PNA-PEG Modified Silicon Platforms as Functional Bio-Interfaces for Applications in DNA Microarrays and Biosensors, ***A. Cattani-Scholz***, *D. Pedone*, *F. Blobner*, *G. Abstreiter*, Technical University Munich, Germany, *J. Schwartz*, Princeton University, *M. Tornow*, Technical University Braunschweig, Germany, *L. Andruzzi*, Ludwig-Maximilians University Munich, Germany

Bio-functional interfaces on semiconductor materials enjoy increasing interest in basic and applied sciences because of the many possible applications of these structures in, e.g., proteomics, micro-array technology and biosensors. For DNA sensing applications single stranded DNA or peptide nucleic acid (PNA) is commonly covalently immobilized via a linker onto the surface which has been pre-modified with a thin organic film before. Here, high hybridization efficiency is generally strived for, together with a maximum suppression of unwanted, nonspecific interactions between target DNA and the sensor surface. We report on the synthesis and characterization of two novel types of PNA interfaces on silicon/siliconoxide substrates featuring poly(ethyleneglycol) $(PEG)_n$ as molecular spacer and backfilling. As type one, phosphonate self-assembled monolayers were derivatized with a 12mer PNA oligomer via modification with and post-functionalization of a maleimide-terminated poly(ethyleneglycol) spacer (PEG_{45}). Similarly, a type two modification consisted of silane self-assembled monolayers which were functionalized with PNA via modification with a maleimide-terminated PEG_{45} spacer and were also subsequently modified with a shorter methoxy-terminated PEG_{12} (back-filling). X-ray photoelectron spectroscopy (XPS) analysis confirmed binding of PEG and PNA to the phosphonate and silane films and indicated that additional PEG chains were tethered to the surface during the backfilling process. We carried out hybridization experiments in the presence of matching and mismatching, fluorescently labeled DNA and found that both types of bio-functional surfaces were effective in the hybridization of matching DNA while significantly reducing non-specific adsorption. To verify the suppression of DNA adsorption on PEG-only modified surfaces and to extend the scheme towards laterally patterned structures we employed micro-molding techniques, i.e., pressed DNA-coated PDMS stamps onto a surface which comprised of alternating PNA functionalized, and non-functionalized regions, respectively, in a grid-like manner. These studies confirmed that hybridization took place selectively at the PNA functionalized regions only, while physisorption at the probe-less PEG-functionalized regions was drastically reduced.

BI-ThP5 Lipid Bilayer Formation and Properties Studied by Combined Electrical Impedance Spectroscopy and QCM-D, ***E. Briand***, *F. Höök*, *B. Kasemo*, *S. Petronis*, Chalmers University of Technology, Sweden

The added value of using synchronized Electrochemical Impedance Spectroscopy (EIS) and Quartz Crystal Microbalance with Dissipation (QCM-D) monitoring is that phenomena and properties, hidden for one of the techniques, may be dynamically resolved by the other one. EIS provides information about the electrical properties of the studied system, while QCM-D provides information about adsorbed mass variations and viscoelastic properties of the adlayer. We have here applied these combined techniques to study (i) supported lipid bilayer formation and (ii) subsequent pore formation using gramicidin D. The results demonstrate how these techniques in combination provide new insights about this and similar bio-adlayer systems. (i) The signatures, produced by the two techniques, of lipid bilayer formation on SiO2 from nanosized POPC liposomes, are quite different. The well established QCM-D signature tells that the initial kinetic phase consists of intact liposome adsorption, followed by vesicle rupture and fusion of lipid bilayer patches to a coherent bilayer. In contrast, EIS does not show any change in impedance until slightly before the critical liposome coverage is reached, where rupture and bilayer formation begins. Furthermore, at the end of the process, where the QCM-D Δf and ΔD signals have reached stable bilayer values, the electrical resistance still varies for several minutes, indicating a rearrangement/annealing process and/or additional minor addition of lipids. The absolute value of the bilayer resistance was found to significantly improve when cations were present in the buffer. (ii) Using a high resistance bilayer as the starting point, the insertion of gramicidin D was followed by QCM-D and EIS. By simultaneously recording EIS signals and changes in the viscoelastic properties (QCM-D) of the bilayer, at different GrD concentrations it was possible to identify the range of concentrations suitable for combined studies of the peptide activity and pore formation.

BI-ThP6 A Simple Method for Making Highly Ordered Chemical Patterns by Sputtering Through Ordered Binary Colloidal Crystals, *G. Singh, V. Gohri, S. Pillai, A. Arpanaei, M. Foss,* ***P. Kingshott***, The University of Aarhus, Denmark

Nanopatterning of biomolecules, such proteins, DNA, and polysaccharides are of great interest in cell culture dishes, biosensors, medical implants and tissue engineering. These so-called nanoarrays require attachment of biomolecules at specific locations on solid substrates with precisely controlled chemistry, but to function fully the non-specific adsorption in surrounding regions must be prevented. Currently, the most widely used techniques for patterning are photolithography, soft lithography, or dip-pen AFM lithography, all of which involve multi-step surface modification directly onto substrates, and are time consuming and expensive. We have shown recently that highly ordered binary polystyrene nanoparticle patterns can be generated from simple self-assembly onto surfaces, where single layers of large particles are surrounded by crystals of smaller particles. Here, we report a novel method for generating chemical nanopatterns by Au sputtering through the crystal layer followed by lift-off of the particles. The crystal regions of the binary pattern, composed of the smaller particles, facilitate transport of the Au sputter beam to the substrate. After particle lift-off only the regions where the small particles have been in contact with the silicon substrate are coated with Au. The large particles act as a mask and remain uncoated, and the thickness of the surrounding Au layer is controlled by the sputter time. The highly ordered chemical patterns are generated where the size of the features are tuned by appropriate choice of particle sizes (50nm to 3µm diameters) and ratios. The stability of the Au layers to aqueous environments is ensured by coating the Si wafer with a thiolated silane, which acts as an adhesion layer. We demonstrate that the resultant Au layer can be coated with a protein resistant mercapto-oligo(ethylene glycol) layer ((1-mercapto-11-undecyl)-tri(ethylene glycol)) that allows selective adsorption of fluorescently labelled proteins on to the Si regions of the pattern. The Au patterns and subsequent protein adsorption are characterized by AFM, SEM and fluorescent microscopy. XPS and ToF-SIMS are used to characterise the chemical modification steps of the patterning. In summary, we introduce a novel method for generating highly-ordered chemical nanopatterns that is very fast, inexpensive, and allows patterns of biomolecules to be created over large areas.

BI-ThP7 Smooth SiO_2 Surface for Biointerfaces Applications Obtained by Oxidation of Polysiloxane Thin Films, ***C. Satriano***, *G.M.L. Messina,* University of Catania, Italy, *S. Svedhem,* Chalmers University of Technology, Sweden, *G. Marletta,* University of Catania, Italy, *B. Kasemo*, Chalmers University of Technology, Sweden

A simple approach for preparation of smooth SiO_2 surfaces by oxidative modification of polysiloxane films is described. Thin films of poly(hydroxymethylsiloxane) (PHMS) were deposited by spin coating on silicon or gold substrates and modified either by radiofrequency oxygen plasmas or combined oxygen plasmas and thermal treatments. The modified films were converted to SiO_x phases, ultimately SiO_2 like, as determined by XPS, and exhibited very high water wettability, as measured by contact angle measurements. Moreover, the high original flatness of the PHMS was not affected by the modification treatments. Both untreated and treated films had roughness values below one nanometer. Using the QCM-D and SPR techniques the adsorption behaviour of and supported lipid membrane (SLB) formation, from small unilamellar vesicles of neutral zwitterionic POPC, positively charged DOEPC and negatively charged DOPC/DOPS mixtures were investigated onto untreated and modified PHMS films. SLBs were successfully obtained on the modified PHMS surfaces. The latter results are compared with corresponding results for PVD deposited SiO_2.

BI-ThP8 Influence of Raft Forming Lipids on Bilayer Formation on SiO_2 Surfaces Studied by Means of QCM-D, ***M. Sundh***, University of Aarhus, Denmark, *S. Svedhem, B. Kasemo,* Chalmers University of Technology, Sweden, *D. Sutherland*, University of Aarhus, Denmark

It is well known that artificial bilayers composed of ternary lipid mixtures of phosphatidylcholine, sphingomyelin (SM), and cholesterol (chol) phase separate and form domains enriched in SM and chol; so called rafts. Rafts are believed to be involved in numerous cellular processes such as cell signaling, endocytosis etc.[1] and thus the importance of their study. Developments in the field of nanotechnology have opened up new routes to the study of molecular systems. The long term goal of these studies involves the use of nanostructured interfaces to systematically define parameters such as membrane curvature and allow the investigation of their correlation to phase separation. As a preliminary step the formation and quality of bilayers, formed with lipid raft compositions, on flat surfaces is investigated. Quartz crystal microbalance with dissipation (QCM-D) is a tool commonly used to study and quantify the adsorption of proteins and the fusion of lipid vesicles into lipid bilayers.[2] In this study the influence of lipid composition in lipid vesicles on the formation of bilayers was investigated and interpreted in terms of the phase separation of the components. Ternary lipid mixtures of POPC/SM/chol at different ratios were formed into unilamellar vesicles by extrusion and deposited on SiO_2 coated QCM crystals. Preliminary results show that the formation of lipid bilayers can be tuned by changing the lipid composition or temperature. An increase in the proportion of SM within the vesicles results in a reduction in quality of the formed bilayer, seen by an increased dissipation response. These results can be interpreted in terms of phase separation into ordered and disordered fluid domains within the vesicles. As the SM concentration is increased the ordered phase becomes dominant up to a point where the vesicles are too rigid to fuse and form bilayers. A temperature study of vesicles with POPC/chol shows that rupture of vesicles could be induced by doing the experiment at increased temperatures and hence changing the lipid phase.

[1] Simons, K. and D. Toomre, Lipid rafts and signal transduction. Nat Rev Mol Cell Biol, 2000. 1(1): p. 31-39.

[2] Reimhult, E., F. Hook, and B. Kasemo, Intact vesicle adsorption and supported biomembrane formation from vesicles in solution: Influence of surface chemistry, vesicle size, temperature, and osmotic pressure. Langmuir, 2003. 19(5): p. 1681-1691.

BI-ThP9 Investigation and Reduction of Current Noise in Solid-State Nanopores, ***D. Pedone***, *M. Firnkes, G. Abstreiter, U. Rant*, Technical University Munich, Germany

Solid state nanopores have emerged as powerful analytical tools to study single molecules. DNA translocation experiments have been conducted with great success in the past, and protein translocation has been demonstrated recently. In these experiments, the biomolecule is detected by measuring the ionic current through the pore, which becomes transiently suppressed when a molecule traverses the pore. The use of solid-state nanopores for studies of complex biomolecular behavior or interactions relies on the ability to record these current blockades with superior fidelity, which poses great challenges with respect to the noise characteristics of the solid-state device. Here we present systematic studies addressing the current noise of solid-state nanopores for translocation experiments. Pores with diameters <10 nm have been fabricated in Si_3N_4 membranes by e-beam lithography and subsequent shrinking in a transmission electron microscope. The electrical characteristics of the nanopore chips in aqueous pH-buffered saline solutions are studied using electrochemical impedance spectroscopy (EIS), cyclic voltammetry, and potential step methods. Equivalent circuit models to represent the nanopore device are proposed based on the obtained data. The frequency dependence of the current noise is recorded with spectral analyzers and discussed within the extracted equivalent circuit models. Within this framework, we investigate the influence of various parameters on the electrical noise: (i) chip designs with different membrane dimensions are realized by combining optical and e-beam lithography with feedback-etching methods, (ii) surface passivation using silicone elastomers and photo-resists are compared, (iii) the composition, salinity, and pH value of the buffer solution is examined. Our results allow us to identify the contribution of various capacitances and dielectric losses across the chip to the measurement noise and suggest guidelines for low-noise translocation recordings.

BI-ThP10 Fabrication and Chemical Surface Treatment of Solid State Nanopores in SiN Membranes, ***M. Firnkes***, *D. Pedone, G. Abstreiter, U. Rant*, Technical University Munich, Germany

Solid state nanopores attracted broad attention in recent years as a tool to study biological molecules like DNA or proteins. In these experiments, the translocation of the molecule through the nanopore is detected by a blockade of the ionic current across the pore. Up to now solid state nanopores are mainly directly drilled into a freestanding silicon nitride membrane via an intense e-beam. Here we report a new pore fabrication technique. Single nanopores are processed in silicon nitride membranes by e-beam lithography and feedback-controlled wet chemical etching, followed by TEM induced shrinking. Moreover we present current noise data showing the influence of various chemical treatments of the pore surface. Starting with a (100) silicon chip of 200 µm thickness, which features 50 nm silicon nitride coatings on both sides, we use optical lithography to form an etch mask on the chip's back side for anisotropic etching of the silicon with KOH. Subsequently we utilize e-beam lithography on the front side to open holes of 40 – 50 nm in the silicon nitride. In the next step the silicon is etched by KOH resulting in a pyramidal shaped undercut of the small holes on the chip front side. During a second KOH etching process from the backside only, we observe the time dependence of the electrical current across the silicon chip. The etching is stopped when a certain current threshold indicates the opening of the pyramid. In this way the pyramid is truncated in a controlled manner. This leads to a 5 x 5 µm freestanding silicon nitride membrane containing the pore. To get the desired pore size we shrink the pores using a TEM. Electrical noise analysis data is presented showing the influence of small membrane sizes resulting from feedback-controlled etching. In addition we studied the influence of the surface termination on wetting properties and electrical noise. In this context we applied both oxidizing (HF) as well as reducing agents (piranha, oxygen

plasma) to change the surface properties of the nanopores. Our results show the benefits of the combination of feedback chemical etching and standard nanopatterning techniques on the electrical noise and indicate how current recordings can be obtained with low noise by a chemical treatment of the nanopore.

BI-ThP11 Determination of Protein Charge with Switch DNA Bio-Sensors, ***J. Knezevic***, *W. Kaiser, E. Pringsheim, G. Abstreiter, U. Rant*, Technische Universität München, Germany

In the recent past, switchable DNA layers have been established as promising candidates for biosensors.[1] Here, the efficiency and dynamics of the electrically induced conformation-switching of surface tethered DNA molecules are used as the sensing parameters. The detection of DNA and proteins (antibodies and antibody fragments) has been demonstrated. Moreover, the size of the captured protein targets could be determined from the switching dynamics on-chip. However, the influence of the proteins' charge remained unclear in these experiments. Here we report on DNA-switching bio-sensing experiments, where the influence of the protein charge was investigated on the basis of the avidin/ streptavidin/ neutravidin-model system. The modulation amplitude of the switching DNA layer was probed electro-optically at low frequencies of the driving electrical signal. The switching kinetics of the tethered molecules were analyzed by frequency resolved measurements. In addition, the double layer charging process was evaluated by impedance measurements. The proteins' charge was varied on-chip by altering the solution pH value. In complementary measurements, the target proteins were characterized regarding their charge and size by dynamic-light scattering. The correlation between the protein charge and size and the low-frequency switching behavior is evaluated. Further, the influence of the protein charge and size on the switching dynamics is described. The results are discussed within the framework of classical electrostatic screening models. Finally, we elucidate the possibility to employ switchable DNA layers for the charge-sensitive detection and characterization of proteins, as well as biomolecules in general.

[1]Rant et al., PNAS 2007, vol. 104, pp. 17364-17369.

BI-ThP12 Surface-enhanced Raman Scattering from Controlled Nanoparticle System, ***S.Y. Chen***, *D.S. Sebba, A.A. Lazarides*, Duke University

We present a theoretical and experimental study of surface-enhanced Raman scattering from core-satellite nanoparticle assemblies of known structure in the solution phase. The detectability of the Raman signal is attributed to enhanced electromagnetic fields localized between plasmonically coupled core and satellite metal nanoparticles. Design of the structures was accomplished using near field calculations based upon Generalized Mie theory, a theory that accurately accounts for multipolar coupling within clusters of spherical particles. Assembly structures are identified on the basis of positions of hot spots and overlap of plasmon resonance frequencies with Raman excitation spectra and available laser lines. Core-satellite structures are assembled using DNA linkers and characterized by transmission electron microscopy (TEM). The control of field strength and plasmon frequency provided by the coupled particle system is expected to provide insight into SERS signaling of use in application of SERS to biomolecule sensing.

BI-ThP13 Magnetic Tweezer Sensor for Ensemble Binding Events of Nonmagnetic Particles, ***R.M. Erb***, *R.E. Ducker, S. Zauscher, B.B. Yellen*, Duke University

Current methods to measure molecular binding strengths include force pulling methods such as atomic force microscopy and optical tweezing. These methods are greatly limited in their throughput, requiring molecular fishing and individual particle targeting that produces binding data on the order of minutes or hours. To overcome these deficiencies, the authors have developed a magnetic system that allows for fast ensemble measurements of thousands of single particle-substrate bonds simultaneously using a High Gradient Magnetic Separation (HGMS) system. The described system is a dense array of micron-size ferromagnetic thin islands on a substrate. These magnetic arrays offer the ability to apply very strong particle forces that can be in the range of tens of nanonewtons, orders of magnitude higher than most optical or electrical systems. Additionally, this system can be used to apply forces on nonmagnetic particles by submerging them in biocompatible magnetic fluids, a technique known as "inverse" magnetophoresis. The ferrofluid causes the nonmagnetic particles to behave as magnetic holes allowing for particle-substrate bonds to be broken through the islands' applied magnetic force. The authors have extended this system onto the surface of a quartz crystal microbalance (QCM) sensor, which allows for the accurate sensing of ensemble particle movement. To test this system, the authors use a mixed self-assembled monolayer of biotin and oligoethylene glycol and selectively bind streptavidin coated particles to the magnetic islands. Using the magnetic islands and an external magnetic field, streptavidin particles are attracted to a preprogrammed edge of the islands and are allowed to undergo molecular binding with the surface. As an opposite external field is applied, the particles will be pulled en masse to the opposite side of the islands, a movement that can be sensed and analyzed by the QCM. Through knowing the applied magnetic forces, this system allows for the ensemble quantification of bond dissociation between any chemically active particle and substrate.

BI-ThP14 Nanoparticle Thermoplasmonic Modulation, *R.H. Farahi*, ***A. Passian***, *A.L. Lereu, T.G. Thundat*, Oak Ridge National Laboratory, *Y. Jones*, Alcorn State University

Single particle thermo-optical properties are increasingly important in applications such as therapeutics, nano devices, and alternative energy sources. In these applications, the temperature dependent electronic characteristics play a role in the feasibility, efficiency, and the functionality of the intended system. We present an investigation of the thermal properties of gold nanoparticles on a quartz substrate using optical excitation of surface plasmons. The surface deformation, in the region of the localized optical modulation of the surface plasmons, is studied as a function of frequency, power, and polarization. A non-linear frequency and power dependence is observed for the nanostructure system as a result of the thermoplasmonic processes including the volumetric deformations. A threshold power for the observation of the modulation is estimated and is in good agreement with theoretical and computational results.

BI-ThP15 Where DNA and Plasmonics Meet- An Investigation into Cooperative Molecular Recognition at a DNA Nanostructure-Metal Interface, ***E.R. Irish***, *T.H. LaBean, A.A. Lazarides*, Duke University

Recent work in assembly of complex DNA nanostructures has demonstrated the effectiveness with which the non-covalent forces of DNA hybridization can drive formation of a topologically rich set of engineered DNA nanostructures. These DNA nanostructures can be used as structural components within a variety of complex nanosystems, including integrated systems for molecular detection. With the advances in the design and solution phase assembly of novel addressable DNA nanostructures, there is a need for the development of new techniques for controlling deposition of the structures on surfaces. The objective of this research is to investigate thermodynamic and kinetic control of interactions between DNA nanostructures and oligonucleotide functionalized gold films. In this research, surface plasmon resonance (SPR) is used for real-time monitoring of the hybridization of DNA structures on oligonucleotide functionalized gold films. Kinetic and thermodynamic parameters derived from the SPR reflectivity data are used to evaluate the effect of multivalence on the strength of interaction. Kinetic measurements, such as the association and dissociation rates, are determined through the monitoring of the SPR response to hybridization as a function of concentration. Ultimately, understanding of the kinetic and thermodynamic parameters that characterize multivalent interactions between DNA nanostructures and gold films will enable engineering of interactions at soft/hard matter interfaces. It is anticipated that the new tools for integrating soft matter on patterned templates will prove useful in future applications of DNA nanostructures that require organization of the soft matter.

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