Friday Morning, October 6, 2000

Biomaterial Interfaces
Room 202 - Session BI-FrM

Biomolecular Recognition at Surfaces

Moderator: K.E. Healy, University of California, Berkeley

8:20am **BI-FrM1 AFM and EM Studies Providing Insights into Membrane Fusion in Cells**, ***B.P. Jena,*** Yale University School of Medicine **INVITED**

Binding force profiles between solubilized synaptic vesicle and synaptosomal membrane components were examined using atomic force microscopy (AFM). These AFM force spectroscopic studies reveal that a 17 nm and a 34 nm long complex form, following interaction between the two sets of membrane components. The formation of such complexes is further confirmed using negative staining electron microscopy (EM), performed on the immunoprecipitated membrane fusion machinery obtained from neuronal tissue. These EM studies performed on the dehydrated immunoprecipitated native fusion complex reveal the presence of 28-30 nm new coiled rod-like structures in association with 14 nm long SNARE complexes. Neuronal SNAREs were found coiled and super-coiled with these structures. The existence of NSF as pentamers in its native state is also demonstrated. The extent of coiling and super-coiling of SNAREs may regulate the potency and efficacy of membrane fusion in cells.

9:00am **BI-FrM3 Adhesion of Mammalian Cells Modeled by Functionalized Vesicles**, ***A. Janshoff,*** *J. Wegener, H.-J. Galla,* WWU Muenster, Germany

Specific molecular recognition between cell-surface receptors and extracellular matrix proteins immobilized on a growth substrate are the most relevant interactions that allow cells to actively spread on a surface. We applied the quartz crystal microbalance technique (QCM) to follow the time course of cell attachment and spreading on artificial substrates. The shift of the sensorÂ´s resonance frequency provides a direct measure for the fractional surface coverage of the piezoelectrically active area. Frequency shifts associated with the establishment of confluent cell layers were found to be dependent on the cell species, reflecting their individual impact on the displacement of the resonator. In order to learn more about the mechanisms that govern the response of shear wave resonators to the attachment of mammalian cells, we modeled the cellular system with unilamellar liposomes doped with biotinylated lipids. Liposome adhesion to avidin/streptavidin coated surfaces was monitored using the QCM technique. Increasing fractions of the biotinylated lipid in the liposome shell resulted in enhanced shifts of the resonance frequency. Concomitant shape analysis of the surface-attached vesicles using SFM revealed an extended contact area between liposome and surface. We conclude, that an increasing number of bonds between the liposome and the surface induces the extended contact areas and that a similar mechanism may be applicable to explain the individual response of the QCM to different cell species.

9:20am **BI-FrM4 PNA-DNA and DNA-DNA Hybridization Detection via Lipid-Biotin-Streptavidin Immobilization on a SiO2 Coated Quartz Crystal Microbalance Sensor**, ***F. Höök,*** *A. Ray, B. Norden, B. Kasemo,* Chalmers University of Technology, Sweden

Surface-based bioanalytical sensors for oligonucleotide hybridization are very attractive for genetic diagnostics, gene therapeutics and the emerging solid phase / real time PCR. Little is however known about how various immobilization strategies affect the conformation and hence function of oligonucleotide strands. We have investigated the possibility to use the dissipative quartz crystal microbalance (QCM-D) technique and controlled surface-immobilization of single stranded synthetic peptide nucleic acid (PNA) as well as DNA, as selective probe(s) for fully complementary and various single mismatch DNA. In order to minimize unspecific binding of DNA, streptavidin was immobilized as a protein 2-D crystal on a biotinylated phospholipid bilayer supported on a SiO@sub 2@ surface in the fluid liquid crystalline phase. This was followed by attachment of a mixed-sequence 15-mer biotin-PNA or a 15-mer biotin-DNA with identical base pairs. The exposure of the streptavidin-immobilized biotin-PNA and biotin-DNA to fully complementary and various mismatch DNA was investigated at 24 °C. Only the fully complementary and singly mismatched DNA oligomers hybridized with the immobilized PNA and DNA, and was possible to discriminate via significant difference in the binding and dissociation kinetics, demonstrating a very high selectively. Most interestingly, however, is that the ratio between hybridization-induced energy dissipation (c.f. rigidity) and the frequency shifts (c.f. mass uptake), allowed us to discriminate different structures of immobilized PNA-DNA and DNA-DNA duplexes. Possible influence on the hybridization kinetics and the structure of the formed duplexes from primarily lateral interaction is discussed.

9:40am **BI-FrM5 DNA Probe Structure and Target Length Effects on Hybridization Kinetics and Efficiency of DNA Self-assembled Monolayers**, ***G.B. Saupe,*** *M.J. Tarlov,* National Institute of Standards and Technology

Optimizing the parameters involved in monolayer DNA hybridization events is important to the emerging DNA sensor array technologies used for a variety of applications including genetic diagnostics, forensics, and infectious disease detection. The objective of our research is to determine how DNA surface coverage, molecular orientation, and sequence identity impact the functionality of DNA array devices. To study these factors we use a model system with short sequences of single-stranded DNA probes self-assembled on gold surfaces through a thiol linker. The gold is also passivated with mercaptohexanol to eliminate non-specific adsorption of DNA to the gold and to enhance the activity of immobilized probes. Surface Plasmon Resonance is used to monitor and derive, in situ, the nanometer-scale thickness changes associated with surface hybridization reactions. Complementary single-stranded DNA targets in high salt buffered solutions hybridize with relatively high efficiency (25-100%) to these surface bound probes. We will report how variations in probe surface structure, the length of ssDNA targets, and the relative position of the complementary sequence in the ssDNA targets affect hybridization kinetics, efficiencies and completion times.

10:00am **BI-FrM6 Probing Protein Interactions with Surface-Immobilized Double-Stranded DNA Using Surface Plasmon Resonance Sensing Techniques**, ***J.S. Shumaker-Parry,*** *C.T. Campbell, K.E. Nelson,* University of Washington*; G.D. Stormo,* Washington University Medical School*; F.S. Silbaq,* University of Colorado*; R.H. Aebersold,* University of Washington

Understanding the molecular mechanisms of gene expression in eukaryotes requires a precise knowledge of the strength and specificity of protein:DNA interactions. Surface plasmon resonance sensing techniques are important for monitoring the adsorption of biomolecules from liquid solutions onto functionalized solid surfaces with high sensitivity and fast time response. Simple methods convert changes in the angle or wavelength at which the surface plasmon resonance (SPR) of a thin metal film is optically excited into adsorbate concentrations. Methods for monitoring interactions between the transcription repressor protein Mnt and surface-immobilized double-stranded DNA using SPR spectroscopy and microscopy will be described. We have immobilized dsDNAs onto a planar gold surface with high density (1-3x10@super 12@ DNA/cm@super 2@, depending on their length) and uniform spacing (~4 nm closest possible DNA-DNA separation). This was accomplished by adsorbing biotinylated DNAs onto a nearly close-packed monolayer of the protein streptavidin prepared first by adsorbing it onto a mixed self-assembled monolayer on gold containing biotin-terminated and oligo(ethylene glycol)-terminated alkylthiolates in a 3/7 ratio. This DNA-functionalized surface resists non-specific protein adsorption. SPR spectroscopy experiments have shown that Mnt binds in 3.8:1 ratio to its immobilized DNA recognition sequence. This is consistent with its behavior in homogeneous solution, where it binds as a tetramer to its DNA binding sequence. A sequence with a single base-pair mutation shows nearly as much Mnt binding, but a completely random DNA sequence shows only 5% of this binding. This proves that DNA-binding proteins bind sequence-specifically to dsDNAs that are immobilized to gold with this streptavidin linker layer. SPR microscopy is being developed to extend these studies to probe protein interactions with an array of dsDNA-containing elements immobilized on a sensor surface.

10:20am **BI-FrM7 Immobilized Antibodies on Functionalized Self-assembled Monolayers: Reactivity, Surface Homogeneity and Microarraying**, ***P. Kernen,*** *F. Zaugg, K. Witte, D. Quincy, P. Indermuehle, S. Nock, P. Wagner,* Zyomyx Inc.

@omega@-Substituted alkanethiols self-assemble in ordered monolayers on Au(111) surfaces and form highly reproducible reactive interfaces for biomolecule immobilization. Reaction conditions for covalent coupling of antibodies to monolayers exposing N-hydroxysuccinimide and other functionalities have been tested using radiometry and fluorescence microscopy. Scanning probe microscopy, X-ray photoelectron spectroscopy and other surface analytical techniques have been applied to characterize homogeneity and surface coverage of covalently bound biomolecules. By incorporating these bioreactive interfaces into microfabricated three-dimensional structures in silicon, functional microarrays of antibodies could be produced with variable feature size.

Author Index

Bold page numbers indicate presenter