Wednesday Afternoon, November 11, 2009

Biomaterial Interfaces
Room: K - Session BI+AS+NS-WeA

Quantitative Nanoscale Sensing at Biosurfaces and Interfaces

Moderator: F. Höök, Chalmers University of Technology, P. Kingshott, Aarhus University, Denmark

2:00pm **BI+AS+NS-WeA1 Characterizing Self-Assembled Supported Lipid Membranes for Biosensing**, ***E.O. Reimhult***, ETH Zurich, Switzerland **INVITED**

More than 50% of all drug targets are membrane proteins, which require a lipid membrane environment to retain correct conformation and function. This highlights the need to create sensing tools for analytical profiling of transmembrane protein function subject to e.g. drug binding. Furthermore, it is increasingly realized that the compositionally complex and dynamically rearranging lipid membranes can be important active regulators of biological function in their own right. The complexity of the *in vivo* cell membrane and the need to apply high throughput techniques like arrays and highly surface sensitive analytical techniques make model systems highly desirable. Thus, supported lipid bilayers (SLBs) which combine control of membrane properties with surface analytical techniques receive increasing interest.

Biosensor interfaces can be easily functionalized with an SLB by self-assembly from liposomes. However, design of more native-like SLBs, e.g., having diverse lipid compositions, including glycolipids or mimics thereof, demands further developments of this assembly technique. This in turn prompts for more advanced characterization of the formation and structure of SLBs.

We present advances in instrumentation and interpretation of data from multi-technique studies of liposome adsorption and SLB formation, which enhance the understanding of the assembly process and the sensor response obtained for different membrane conformations. In particular, we demonstrate advances in waveguide spectroscopy which allow for characterization of the rupture kinetics of supported lipid bilayers by liposome fusion, but also to in real time distinguish differences in structure for membranes of different compositions and under various environmental conditions. These advances also open the possibility to study differential binding to and into SLBs and to use rearrangements in the SLB as an amplifier of membrane protein binding events.

As examples, we also present the results of such detailed multi-technique characterization of the self-assembly of new supported lipid membrane mimics, e.g., bacterial membrane mimics containing lipopolysaccharides and poly(ethylene glycol)-lipids, including how the presence of a polymer directly attached to the lipids affects the self-assembly and how the hydrophilic polymer is distributed and rearranged in the membrane under mechanical perturbation. Such self-assembled polymer-membranes have great potential for creation of membrane arrays incorporating membrane proteins thanks to high stability and less perturbation of the membrane components due to the mobile polymer spacer layer.

2:40pm **BI+AS+NS-WeA3 Nanoplasmonic Biosensing: Artificial Cell Membranes, Structural Changes and Quantification of Bound Mass**, ***M. Jonsson***, *A. Dahlin, P. Jönsson, S. Petronis, F. Höök*, Chalmers University of Technology, Sweden

The resonance condition for excitation of plasmons associated with metal nanostructures is highly sensitive to changes in the interfacial refractive index, which has made the phenomenon highly popular as transducer principle for label-free sensing of biomolecular recognition reactions. There is a particular need for sensor concepts that are compatible with studies of the cell membrane, which can be explained from the fact that more than half of the most commonly used drugs are directed towards membrane-associated reactions. This is also relevant with respect to diagnostics of viral diseases, because viruses typically infect host cells via adsorption to the cell membrane. During the past years we have developed nanoplasmonic biosensing platforms that are compatible with studies of artificial cell membranes, such as lipid vesicles and supported lipid bilayers (SLBs).[1-3] In addition to probing specific binding of ligands to membrane receptors, we showed that nanoplasmonic sensors provide a unique means to probe biomolecular structural changes, such as during the formation of a SLB from adsorption and rupture of lipid vesicles.[1]

We have previously used a metal film perforated with nanoholes as an electrode for combined nanoplasmonic and quartz crystal microbalance measurements.[3,4] Besides two independent measures on biomolecular structural changes, the combined sensor setup was shown to provide new information that enabled the quantification of adsorbed mass on the sensor surface with only the density of the molecules as unknown parameter.[3]

In the current work we utilize the continuity of a perforated plasmon active metal film to fabricate nanoplasmonic pores with liquid access to both sides of the nanopores.[5] This structure opens up for a wide range of novel applications. For example, extending our previous work on plasmonics and cell membrane mimics, an appealing possibility is to measure transport of both charged and non-charged molecules through lipid membranes that span the pores. Plasmonic pores can also be used for flow-through sensing, where flowing the target molecules through the pores will facilitate molecules to reach the sensor surface in an efficient way and circumvent limitations due to mass-transport.[6]

References:

1 Jonsson, M. P. et al. *Nano Letters* **2007**, *7*, 3462-3468.

2 Dahlin, A. B.; Jonsson, M. P.; Höök, F. *Advanced Materials* **2008**, *20*, 1436-1442.

3 Jonsson, M. P.; Jönsson, P.; Höök, F. *Analytical Chemistry* **2008**, *80*, 7988-7995.

4 Dahlin, A. B.; et al. *ACS Nano* **2008**, *2*, 2174-2182.

5 Jonsson M. P. et al. Manuscript in preparation

6 Eftekhari F. et al. *Analytical Chemistry* **2009**, ASAP

3:00pm **BI+AS+NS-WeA4 Transfer of Biomolecules between Lipid Membranes**, ***A. Kunze***, *S. Svedhem*, Chalmers University of Technology, Sweden, *P. Sjövall*, SP Technical Research Institute of Sweden, *B.H. Kasemo*, Chalmers University of Technology, Sweden

The study of the interaction between biomembranes is of great interest for both basic research and applications in biosensing technology. In biological systems the interaction between membranes including transfer of biomolecules plays a pivotal role. For instance, it is central in energy supply to and communication between cells and for the function of a large number of drugs. A controlled transfer of lipid molecules, or other biomolecules, between lipid vesicles (liposomes) and solid supported lipid bilayers (SLBs) provides a new platform for modifying and controlling SLBs that can be used in biosensing technology. Mechanistic studies of this process are furthermore important for the understanding of a number of important biomolecule-membrane and inter-membrane events.

We will present how transfer of biomolecules between an SLB and liposomes can be monitored in real-time giving more insight into the complex mechanism of transfer including influence of electrostatic interaction, ionic strength, phase and molecular structure of lipids, as well as time scale of the transfer process. Recent results show that the interaction process consists of an attachment-transfer-detachment (ATD) sequence, where added liposomes first attach to a preformed SLB, then transfer lipid molecules and eventually detach, leaving behind a compositionally modified SLB and ditto vesicles.[1] We will demonstrate how the ATD process can be used for *in situ* modifications, changing the membrane composition, e.g. for the formation of a highly stabilized (SDS-resistant) lipid monolayer on TiO_2, which can then be used for the reassembly of an SLB.[2] We propose this as a promising method for *in situ* preparation of asymmetric SLBs.

The main experimental techniques used to study these processes at these interfaces between two biomembranes are the quartz crystal microbalance with dissipation monitoring (QCM-D), t otal internal reflection fluorescence microscopy (TIRF), fluorescence microscopy and time-of-flight secondary ion mass spectrometry (TOF-SIMS) and optical reflectometry.

[1] Kunze, A.; Svedhem, S.; Kasemo, B. Lipid Transfer between Charged Supported Lipid Bilayers and Oppositely charged Vesicles, Langmuir in press

[2] Kunze, A.; Sjövall, P., Kasemo, B.; Svedhem, S. In situ preparation and modification of supported lipid layers by lipid transfer from vesicles studied by QCM-D and TOF-SIMS, J. Am. Chem. Soc., 131:2450-2451, 2009

4:00pm **BI+AS+NS-WeA7 Nanopores for Sensing Membrane Processes and Enzyme Reactions**, ***M. Mayer***, University of Michigan **INVITED**

This talk demonstrates that pores with diameters below 50 nanometers make it possible to detect enzyme reactions, molecular phases transitions, and nanoscale self-assemblies *in situ* and in real time. For instance, coating the inner walls of nanopores with self-assembled lipid bilayers, afforded controlled shrinkage of this pore to a size that made it possible to detect individual proteins. Remarkably, the extent of pore shrinkage could be

controlled with sub-nanometer precision by the chain lengths of the acyl chains on the lipids that were chosen to assemble the bilayer. Due to the extreme sensitivity of single-channel recording of ion currents through nanopores, this approach made it possible to monitor molecular changes and rearrangements of the lipid bilayer. These changes included phase transitions, variations in membrane composition, and enzymatic reactions on membranes. For example, this approach made it possible to monitor the activity of attomolar amounts of phospholipase D (PLD) and phospholipase C (PLC) – two membrane-active enzymes that are critical for cell signaling.

4:40pm **BI+AS+NS-WeA9 Development of Microresonator Arrrays for Mass and Viscoelastic Characterization of Adsorbed Molecular and Biomolecular Thin Films**, ***D.L. Allara***, *S. Tadidagapa, P. Kao*, Pennsylvania State University

A multiple pixel micromachined quartz crystal resonator array with a fundamental resonance frequency in the 60-100 MHz range has been designed, fabricated, and tested for applications to accurate mass and viscoelastic measurements of adsorbed thin molecular and biomolecular films. Operating with high Q-factors in the range of 25000–50000 and appropriately lower in liquids, the high stability and inherent low noise of the quartz crystals allow for an unprecedented resolution of one part in 10 million for density/viscosity variations. Further, multiple pixels, capable of independent functionalization with SAMs, can be tracked in parallel to give large numbers of independent measurements simultaneously. By measuring the frequency decrease at overtone frequencies it also is possible to vary the decay length of the shear wave away from the electrode and thereby identify individual variations in the density and viscosity of the local environment and accurately monitor small changes in the viscoelastic loading of adsorbed films. The performance of the resonator is illustrated with examples such as the adsorbed protein films in which the damping factor undergoes an order of magnitude change in transitioning from monolayer to multilayer adsorption. This aspect is highly desirable for accurate determination of behavior such as conformational changes.

5:00pm **BI+AS+NS-WeA10 Plasmonically Coupled Nanoparticle-Film Molecular Ruler**, ***R.T. Hill***, *J.J. Mock, A. Degiron, S. Zauscher, D.R. Smith, A. Chilkoti*, Duke University

Experimental analysis of the plasmonic scattering properties of gold nanoparticles controllably placed nanometers away from a gold metal film shows that the spectral response of this system results from the interplay between the localized plasmon resonance of the nanoparticle and the surface plasmon polaritons of the gold film, as previously predicted by theoretical studies. In addition, the metal film induces a polarization to the single nanoparticle light scattering resulting in a doughnut-shaped point spread function when imaged in the far-field. Both the spectral response and the polarization effects are highly sensitive to the nanoparticle-film separation distance, and thus, the plasmonically coupled NP-Film system represents a new variant of the previously reported plasmonic molecular rulers. A surface-based molecular ruler shows promise in potential biosensor and diagnostic devices.

5:20pm **BI+AS+NS-WeA11 Label-free Imaging of Cell Adhesion Dynamics using Surface Plasmon Resonance Imaging Ellipsometry**, ***D.W. Moon***, *J. Gil, W. CheGal, H. Cho, S. Kim*, Korea Research Institute of Standards and Science, S. Korea

The interaction between cell and extracellular matrix (ECM) governs multiple cellular functions and contributes to promote inflammation and tumor metastasis. Therefore, cellular behavior needs to be monitored in the ECM interactive circumstance. Most of previous studies on cell adhesion are based on immunofluorescence microscopy. For cell adhesion dynamics studies, label-free optical techniques that can monitor continuously cell-ECM interfaces for living cells are required.

Here we developed surface plasmon resonance imaging ellipsometry (SPRIE) which can simply image cell-ECM interfaces for live cells with high contrast and at real-time. To visualize cell adhesions to ECM, null-type imaging ellipsometry technique with the attenuated total reflection coupler was applied and both of transverse magnetic and electric waves were made use of. These characteristics make it possible to acquire the high contrast image of cell adhesions. Different features and dynamics of cell adhesion patters in ~ 100 nm cell-ECM interfaces were observed for A10, human coronary artery smooth muscle cell hCASMC, and human umbilical vein endothelial cells (HUVEC) on fibronectin and collagen ECM layers with 1 μm spatial resolution and 30 sec time interval upto 3 days. Harmonized changes of entire adhesion proteins were observed during cell division and cell migration through our imaging system without any labeling. SPRIE images were compared with confocal fluorescence microscopic images of cell adhesion proteins for validation of SPRIE images. Preliminary results on SPRIE studies on the effect of shear force on cell adhesion and migration will be also discussed.

We expect that SPRIE cell adhesion dynamic imaging methods would be useful for further understanding of cell biology and development of drug screening methodology relevant to cell adhesion and migration.

5:40pm **BI+AS+NS-WeA12 Label-Free Determination of Protein-Ligand Equilibrium in Aqueous Solution using Overlayer Enhanced Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (OE-ATR-FTIR)**, ***T.C. Ruthenburg***, *S.S.N. Park, T.A. Aweda, C.F. Meares, D.P. Land*, University of California, Davis

Protein binding/affinity studies are often performed using Surface Plasmon Resonance techniques that don't produce much spectral information. Measurement of protein binding affinity using FTIR is traditionally performed using high protein concentration or deuterated solvent. By immobilizing a protein near the surface of a gold-coated germanium internal reflection element interactions can be measured between an immobilized protein and small molecules in aqueous solution. Using flow injection analysis the on and off rates of these interactions and dissociation constant for the system can be determined. The dissociation constant for the molecule Yttrium-aminobenzyl-DOTA binding to the antibody 2D12.5 system was determined.

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