Thursday Morning, October 18, 2007

Biomaterial Interfaces

Room: 609 - Session BI-ThM

Biomimetic Phospholipid Interfaces

Moderator: F. Höök, Lund University, Sweden

8:00am **BI-ThM1 Surface-supported Bilayer Platforms for Fundamental Research and Biotechnological Applications**, ***K. Hristova***, Johns Hopkins University **INVITED**

Approximately 20% of the open reading frames in complex organisms encode membrane-associated proteins. Despite their abundance and key roles in cell adhesion, recognition, motility, energy production, transport of nutrients and cholesterol, our knowledge of the folding and the structure of membrane proteins is limited, and lags far behind that of soluble proteins. In part, this is due to limited biophysical tools to adequately probe the physical-chemical principles underlying membrane protein function. In our laboratory we have developed a model surface-supported bilayer platform, based on a directed assembly approach that overcomes current limitations associated with traditional black lipid membranes and self-assembled membrane mimetics. The behavior of the proteins in the surface-supported bilayer, as determined by their sequence, is not altered by the assembly method. Thus, the platform is suitable for biophysical characterization of membrane proteins and can be used as a tool to probe diffusivity, secondary structure, thermodynamics of interactions, and electrical response. The long-term goal is to use the platform for biotechnological applications, including drug screens and analyte sensing.

8:40am **BI-ThM3 Supported Lipid Structures as Model Systems for Membrane Associated Interactions**, *S. Svedhem*, ***A. Kunze***, *E. Briand, A. Wikström, B. Seantier, P. Axelsson, H. Ekstrand, M. Edvarsson, S. Petronis, M. Zaech, B. Kasemo*, Chalmers University of Technology, Sweden

Engineering of surface-supported lipid membrane model systems is currently a very active field of research. The present contribution will present a number of recent examples in this area from our group, including both different kinds of supported lipid structures; supported lipid bilayers and vesicles, tethered vesicles, and hole-spanning membranes; as well as different kinds of biomolecular interactions associated with them. The main techniques used to follow these processes are the quartz crystal microbalance with dissipation monitoring (QCM-D), surface plasmon resonance (SPR), optical reflectometry, fluorescence microscopy, and atomic force microscopy (AFM). Some new findings about the factors influencing how lipid bilayers or monolayers of intact vesicles are formed on SiO2 surfaces, e.g. with respect to cations present in the buffer, will be presented. We will also describe new results about processes on the suported lipid structures. In particular, the action of different kinds of lipases (via hydrolysis of phospholipids) on supported lipid bilayers has been monitored by different techniques, as well as the effect of lipases on tethered vesicles (including PEG-ylated vesicles of relevance for drug delivery applications). Another example that will be covered is the exchange of lipid material between charged supported lipid membranes and vesicles; potentially a method for the in situ modification of supported membranes.

9:00am **BI-ThM4 Anisotropic Diffusion in Nanopatterned Supported Lipid Bilayers**, ***J. Tsai***, *E. Sun, J.C. Hone, L. Kam*, Columbia University

Membrane-associated proteins have a central role in cell-cell adhesion and communication, mediated in part by the ability of membrane proteins to diffuse along the cell surface. Many of these proteins exhibit long-range (tens of micrometers) diffusion coefficients that are orders of magnitude smaller than that expected for membrane components. Moreover, other experiments suggest that over short (submicrometer) distances, the diffusive properties of these proteins more closely resemble that of membrane lipids. To capture this anomalous diffusion in a controllable, in vitro model, glass-supported lipid bilayers were patterned with nanoscale barriers of chromium and/or titanium, creating periodic barriers that mimic the spacing of cytoskeletal elements in cells (which underlie several models of anomalous diffusion). Specifically, these barriers consisted of 50-nm wide, parallel barriers spaced at 125 and 250 nm intervals. Gaps in these barriers, measuring 30-50 nm and spaced at 500 nm intervals, were introduced to allow a limited amount of long-range diffusion across the barriers. Long-range diffusion coefficients of Texas-Red-DHPE, in a background of vesicles of Egg PC, were measured using an image-based, fluorescence recovery after photobleach approach. The long-range diffusion coefficient of lipids parallel to the barriers was similar to that on non-patterned glass for both types of metals and all geometries. In contrast, long-range diffusion perpendicular to the barriers was decreased by as much as a factor of ten, dependent on the pattern geometry. Barrier spacing, rather than gap size, was the major determinant of long-range diffusion. Barrier material had an additional influence. On surface with chromium lines, photobleach recovery agreed with a model of diffusion along a perforated surface, suggesting that this material forms perfect barriers to lipid diffusion. On surfaces patterned with titanium, diffusion across the barriers was consistently higher than predicted by the model; furthermore, lipids exhibited limited diffusion across barriers with no gaps. These results suggest a more complex interaction between the supported lipid bilayer and the substrates. The nature of this interaction is currently under investigation. In summary, we describe a controllable, nanopatterned supported lipid bilayer model that captures the complex patterns of membrane protein diffusion, which have immediate use in the study of cell-cell communication.

9:20am **BI-ThM5 Fabrication of Surface-Engineered 3D Micro-Well Arrays for High Resolution and High Throughput Analysis in a Single Cell Format**, ***M. Textor***, *M. Ochsner, M. Smith, H.M. Grandin, S. Luna-Morris, V. Vogel*, ETH Zurich, Switzerland

In addition to substrate rigidity, matrix composition, and cell shape, dimensionality is now considered an important physical property of the cell microenvironment which directs cell behavior. However, available tools for the study of cell behavior in two-dimensional (2D) versus three-dimensional (3D) environments are difficult to compare, and no tools are available which provide 3D shape control of individual cells. Using microfabrication and replication techniques, we developed PDMS substrates for the culture of single cells in 3D arrays compatible with both high-resolution microscopy and high-throughput analysis. Cell adhesion was limited to within microwells by passivation of the flat upper surface through 'wet-printing' of a non-fouling polymer and backfilling of the wells with either specific adhesive proteins or lipid bilayers, the latter produced by exposing the oxidized PDMS wells to lipidic vesicles that spontaneously fused to form supported lipidic membranes. The surfaces were characterized at each fabrication stage by confocal laser scan microscopy (CLSM) in conjunction with labeled polymers and proteins, and the Quartz Crystal Microbalance (QCM-D) technique and FRAP to characterize bilayer formation on PDMS surfaces. Endothelial cells constrained within microwells were viable, although cell death was increased in very constrained microwells as has been reported for cells on flat substrates. In contrast to studies on 2D surfaces, actin stress fibers were present even within cells cultured in very constrained microwells, and in addition the cytoskeleton was 3D and not limited to the cell-substrate interface. These observations demonstrate that microwells can be used to produce microenvironments for large numbers of single cells with 3D shape control and can be added to a repertoire of tools which are ever more sought after for both fundamental biological studies as well as cell-based assays for drug development and screening. Future work is directed towards the study of (stem) cell differentiation and its dependence on surface (bio)chemistry, ligand mobility and substrate rigidity as well as the development of microwells with walls covered by cadherin-functionalized lipidic membranes simulating a microenvironment that is possibly closer to the one of cells in multicellular colonies and tissue.

9:40am **BI-ThM6 Effects of Fluidity on HIV-1 Neutralizing Antibody Binding to Membrane Surfaces**, ***Y. Lam***, *W. Goo, S.M. Alam, S. Zauscher*, Duke University

Recent studies show that HIV-1 may take advantage of the phenomenon that healthy individuals do not normally produce self reactive antibodies. Broadly neutralizing monoclonal antibodies (nAbs) 4E10 and 2F5 bind to epitopes in the membrane proximal external region (MPER) on the HIV-1 transmembrane envelope glycoprotein (Env) gp41. Unlike most antibodies, however, they also react with several common membrane phospholipids. This auto-reactive characteristic may explain why they are rarely (if ever) found in HIV-1 patients. Little is known about the mechanics of these interactions between nAb and membrane. Initial surface plasmon resonance (SPR) studies suggest a two phase binding model where the nAb first encounters the membrane epitope proximal region, and then docks more securely in a second step. One theory for the mechanism behind this model suggests nAb may first bind to lipid, then diffuse on the lipid until encountering the MPER region on Env gp41. In our work, we investigate the effects of membrane fluidity in this process. Increasing temperature adversely affects this interaction, as it increases the on-rate, but more rapidly increases the off-rates. In order to eliminate rate effects, we monitor protein binding to peptide sequences embedded in mobile supported lipid bilayers and in static lipid monolayer surfaces. Quartz crystal microbalance with dissipation monitoring (QCM-D) was used to verify bilayer and

monolayer formation as well as to measure nAb binding. This knowledge will provide insight into the mechanism of nAb-lipid binding, which may facilitate the production of effective HIV-1 therapies.

10:00am **BI-ThM7 Rupture, Spreading, and Healing of 2D Fluid Lipid Bilayers at Chemically and Topographically Structured Surfaces**, ***A. Parikh***, University of California, Davis **INVITED**

Interfacial organization of lipids and amphiphiles into a discrete number of molecular layers provides, arguably, one of the most pristine experimental realizations of self-organized, two-dimensional systems. it provides an experimental test-bed for the study of a rich variety of interface-dominated processes, including surface melting, low-dimensional phase transitions, surface dynamics, and phase coexistence and separation. This talk will present recent experimental evidence from our laboratories which highlight the importance of substrate structure (e.g., topography, charge, and surface energies) in influencing the dynamics of formation of interfacial single lipid bilayers and their equilibrium morphologies. This work is performed in collaboration with Babak Sanii, Michelle Smith, Alan Szmodis, Adrian Brozell, and Michael Howland. The work is supported by a grant from U.S. Department of Energy.

10:40am **BI-ThM9 Activity of Transmembrane Protein Maintained in Polymerized Lipid Membranes**, ***H. Zhang***, University of Arizona, *V. Subramaniam*, Univeristy of Arizona, *S. Burnside*, *G. D'Ambruoso*, *M. Brown*, *S. Saavedra*, University of Arizona

Membrane-associated proteins are of great importance in transport and signal transduction cascades and therefore are crucial pharmacological targets. With the goal of developing biosensors to screen pharmaceutical candidates, numerous research groups have reconstituted transmembrane proteins into lipid membranes, which provide a biocompatible environment. Our group has been investigating the use of synthetic lipids that can be polymerized to form a highly stable bilayer. Absorbance spectroscopy and plasmon waveguide spectroscopy (PWR) are being utilized to monitor the photoactivity of a G-protein coupled receptor (GPCR), bovine rhodopsin, in both polymerized liposomes and planar supported lipid bilayers (PLSBs), respectively. The photoactivity of rhodopsin is largely retained in some types of poly(lipid) membranes. However, the lipid structure and bilayer polymerization clearly affect the retention of protein activity. PWR is used to probe conformational changes accompanying rhodopsin photoactivation in PSLBs, whereas absorbance spectroscopy is used to directly measure formation of the rhodopsin activated state. Studies are also being performed to investigate the effect of lipid structure on the activity of other types of GPCRs, e.g. the human delta opioid receptor.

Authors Index

Bold page numbers indicate the presenter