Monday Morning, October 2, 2000

Biomaterial Interfaces

Room 202 - Session BI+SS-MoM

Biological Surface Science

Moderator: D.G. Castner, University of Washington

8:20am **BI+SS-MoM1 Suspended Lipid Bilayers on Porous Alumina Surfaces**, ***C. Steinem***, *J. Drexler, C. Hennesthal,* WWU Muenster, Germany

The study presents a new class of artificial membrane system "suspended bilayers" closing up the gap between solid supported membranes (SSMs) and black lipid membranes (BLMs). Suspended bilayers were prepared on the basis of porous alumina surfaces which were produced by an anodic etch of neat alumina foils. Gold was evaporated on the upper surface of the porous material. The porous material was characterized by scanning electron microscopy (SEM), scanning force microscopy (SFM) and impedance spectroscopy. As revealed by SEM and SFM pores exhibit a mean diameter of 65 nm. Functionalization of the upper gold surface was achieved by self assembly of 3-mercaptopropionic acid (MPA) rendering the surface negatively charged at pH 8.6. To obtain suspended solid supported we fused unilamellar vesicles of N,N-dimethyl-N,N-dioctadecyl ammonium bromide varying in their sizes on the MPA-covered surface. Bilayer patches spanning the pores were visualized by scanning force microscopy in buffer using contact mode. The bilayer covered nanopores are thought to serve as second aqueous compartments of femtoliter volume providing enough space to incorporate transmembrane proteins and to generate ion gradients across the membrane together with the ability to use sensitive surface analysis tools.

8:40am **BI+SS-MoM2 Formation and Characterization of Stabilized Supported Phospholipid Bilayers**, ***S. Saavedra***, *E. Ross, J. Conboy, S. Liu, D.F. O'Brien,* University of Arizona

The utility of phospholipid bilayers as non-fouling coatings in molecular device technologies is hampered by the chemical and mechanical instability of these structures relative to (for example) alkylsiloxane self-assembled monolayers. Towards the use of lipid bilayers in applications such as biosensing, we have investigated two-dimensional polymerization as a strategy to stabilize planar supported lipid bilayers. UV-induced and redox-initiated polymerization have been used to prepare air-stable bilayers from phosphatidylcholine monomers containing sorbyl moieties in the acyl chains. Preparation of these structures using Langmuir-Blodgett-Kuhn and vesicle fusion techniques, and characterization of their physical and chemical properties, including nonspecific protein adsorption behavior, will be described.

9:00am **BI+SS-MoM3 Regulating Molecular Recognition and Self-assembly via Mechanical Forces: The Cell Adhesion Protein Fibronectin at Phospholipid Interfaces**, *A. Krammer, G. Baneyx, D. Craig,* University of Washington*; K. Schulten,* University of Illinois at Urbana-Champaign*;* ***V. Vogel***, University of Washington **INVITED**

While major progress has been made in the past to reveal how chemical factors regulate biorecognition, insight into pathways by which nature utilizes external forces to regulate biorecognition and signaling holds the potential for major new discoveries in biomedicine. Knowledge in this field is rudimentary since high-resolution crystallographic structures of biomolecules have mainly been obtained from equilibrated states. The role played by mechanical forces applied to the terminal ends of domains in regulating exposure of their recognition sites will be discussed here for the multidomain protein fibronectin. One of fibronectins many functions is to promote cell adhesion to surfaces. Starting from the equilibrium structure of fibronectin type III domains (FnIII), steered molecular dynamics simulations were applied to study the pathway by which their tertiary structures unravel under external forces. First we found that the accessibility of the cell recognition site on the FnIII10 domain, i.e. the RGD-loop, to integrins is reduced in an early stage of the forced unfolding pathway. Furthermore, forced unfolding studies of various fibronectin type III modules have shown that FnIII-7, FnIII-8, FnIII-9 and FnIII-10 differ considerably in their mechanical stability, and the simulations predict that FnIII-10 unfolds first. Finally, we have experimentally analyzed the pathway on which fibronectin assembles into fibrillar networks underneath phospholipid monolayers, and find again that mechanical forces are crucial to initiate its spontaneous self-assembly. Thus, spontaneous assembly of fibronectin into fibrils cannot be induced by adsorption to solid surfaces, yet it is the fibrillar state that allows cells to apply the forces needed to partially unfold fibronectin's domains.

9:40am **BI+SS-MoM5 New Methods for Patterning Fluid Lipid Bilayer Membranes on Solid Supports**, ***J.S. Hovis***, *S.G. Boxer,* Stanford University

Two new methods are introduced for patterning fluid lipid bilayer membranes on solid supports. These methods, called blotting and stamping, rely on the observation that supported lipid bilayers exhibit self-limiting lateral expansion. The consequence is that it is possible to pattern these fluid surfaces without modifying the underlying substrate. Together these methods constitute a simple and powerful approach for preparing patterned fluid lipid bilayers in nearly any geometry. One important application of these methods is the ability to create composition arrays of lipids and membrane associated proteins. These arrays allow the opportunity to study lipid-lipid, lipid-protein, and/or protein-protein interactions in a parallel fashion. Information gained about these important biological interactions will be highlighted.

10:00am **BI+SS-MoM6 The Interaction of Phospholipid Vesicles with Binary Alkanethiol/Hydroxythiol Monolayers**, ***V. Silin***, National Institute of Standards and Technology*; H. Wieder,* Max Planck Institute for Polymer Research, Germany*; J. Woodward, A. Plant,* National Institute of Standards and Technology

Surfaces modified by self-assembly have applications in sensors, diagnostics, chemical processing, and biomaterials, where they may incorporate features such as molecular recognition and enzymatic activity. Understanding the forces that direct self-assembly of biologically important molecules in predictable arrangements will aid the development of such applications. The focus of this study is a mimic of biological membranes formed by the interaction between two self-assembled systems: phospholipid amphiphiles that associate into bilayer vesicles in water, and monolayers of alkanethiols on metal surfaces. We have studied the interaction of small (60nm) POPC vesicles with binary thiol monolayers of known surface free energy. The surfaces were prepared on gold by self-assembly from binary solutions of the thiols CH3-(CH2)10-X (X = CH3; OH) in THF. The surface plasmon resonance (SPR) technique was utilized to follow the vesicle fusion kinetics and to characterize the resulting assemblies. A dramatic influence of the surface layer composition on the formation of POPC films was observed. The formation of an additonal POPC monolayer was detected only on the completely hydrophobic (100% CH3) surface. The largest thickness of POPC layer was detected at a CH3/ OH ratio of 50% (in the assembly solution). For the completely hydrophilic surface (100 % OH) the POPC layer thickness was found to be close to the thickness of a phospholipid bilayer. Thus, the increase of hydrophilic component on the surface leads to the formation of an unordered POPC film that seems to contain a mix of fused and unfused vesicles. Most likely the formation of an ordered bilayer of POPC molecules has been observed for the completely hydrophilic surface. The SPR data were supported by AFM, capacitance and contact angle measurements.

10:20am **BI+SS-MoM7 Formation of 2D Crystals of Proteins on Solid Supports, and Their Application for Immobilizing Molecules or Particles**, ***A.D.R. Brisson***, University of Groningen, The Netherlands **INVITED**

The immobilization of molecules or particles on solid supports constitutes a central issue in various fields, eg. the immobilization of enzymes in the biosensor area, or the immobilization of DNA molecules on microarrays in genomics. Existing technologies rely mainly on the chemical modification of solid surfaces and the subsequent immobilization of the molecules of interest via non-specific interactions. The strategy we have selected is based on the use of functionalized 2D crystals of proteins formed on solid-supported lipid bilayers (SPBs) as a matrix for anchoring proteins/particles in a specific manner. Its main potential advantages are the wide panoply of functional groups that could be introduced in proteins, the well-known chemistry of the coupling reactions involved, the well-defined density of anchoring groups, and the specificity of the coupling reactions ensuring an oriented binding of bound molecules. In addition, protein 2D crystals could serve as templates for creating ordered arrays of immobilized particles, at the nanometer scale. The formation of SPBs by fusion of lipid vesicles on mica,@footnote 1@ and the growth of protein 2D crystals on SPBs were extensively studied by AFM and Electron Microscopy (EM) in the case of two protein systems, annexin V@footnote 2@ and streptavidin. Pre-formed 2D arrays of modified annexin molecules were used for immobilizing proteins, liposomes, and membrane fragments containing membrane proteins. An unexpected result was the induced ordering of membrane proteins resulting from their specific binding to an ordered protein matrix. On the other hand, while close-packed assemblies of liposomes could be bound to protein 2D arrays, attempts to fuse them into suspended lipid bilayers have yet been unsuccessful. The immobilization of

inorganic particles is under investigation. Results of these studies will be presented. @FootnoteText@@footnote 1@Reviakine et al. Langmuir 16, 2000, 1806. @footnote 2@Reviakine et al. J. Struct. Biol. 121, 1998, 356.

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