Biomaterial Interfaces
Room 312 - Session BI2-WeM

Biomembranes and Spectroscopy
Moderator: J. Hickman

8:20am **BI2-WeM1 Fabrication of Well Structures with Electrode by Synchrotron Radiation Etching and Formation of Lipid Bilayer Giga-Ohm Seals**, ***Md. Rahman,*** The Graduate University for Advanced Studies, Japan*; R. Tero,* NINS, Japan*; Y.-H. Kim,* The Graduate University for Advanced Studies, Japan*; T. Yano, M. Aoyama,* NINS, Japan*; R. Sasaki, H. Nagai, M. Yoshida,* AISHIN SEIKI Co., Ltd., Japan*; T. Urisu,* NINS, Japan

Supported membrane is a lipid bilayer supported on solid surfaces, and is useful as an artificial cell membrane for the study of biological reactions of membrane proteins. We are developing supported membrane biosensors for the purpose of developing the new research tool of the cell membrane surface reactions. These devices are interesting also from the view point of the application to the large scale screening method for the new medicine development. We have established a technique to fabricate a well-type microelectrode with about 1 μm diameter on the surface of a SiO@sub 2@/CoSi@sub 2@/Si substrate. The SiO@sub 2@/CoSi@sub 2@/Si was covered by Co contact mask by sputtering deposition. The circle pattern was made on the Co mask using the femto-second laser ablation. The SiO@sub 2@ was etched by synchrotron radiation (SR) etching. SR was used because of its unique features such as high spatial resolution, extremely high material selectivity between CoSi@sub 2@ and SiO@sub 2@, low damage, and clean etching atmosphere. AFM images of the SiO@sub 2@ surface after the removal of the Co mask with 0.1 M HNO@sub 3@ aq. showed that the surface was very flat (R@sub a@=0.8 nm). Ag (50 nm) was deposited by electroplating on the CoSi@sub 2@ which is exposed at the bottom of the etched well. Then the surface of the Ag was coated with AgCl also by electroplating. A single planar lipid bilayer (DPPC : POPS = 90:10) was deposited on these microelectrodes by the rapture of giant unilamellar vesicles. From the I-V characteristics of the membrane, the resistance of the lipid bilayer was 1.2 G@ohm@. This value was larger enough for the single channel current measurement. The measured capacitance was 10.7 pF, which is larger than that estimated from the total electrode area. This may be due to that the charge is accumulated at the larger area of the lipid bilayer than the area of just the electrode holes by the current through the thin (1 nm) water layer under the lipid bilayer.

8:40am **BI2-WeM2 Probing Lipid Membrane Responses to Surface Morphology**, ***S.D. Gillmor,*** *J.J. Heetderks, X. Wang, Q. Du, P.S. Weiss,* The Pennsylvania State University

The outer cellular membrane is a mixture of protein receptors, lipids and cholesterol whose organization is incompletely understood. Many cell membranes have complex interactions with the underlying basement membrane, and our investigations focus on lipid deformation due to adhesion to this support. We model the complex basement membrane structure chemically and topographically, through lithographically defined features for control over substrate morphology, and through chemical modification of the surface. Initially using simplified, lipid-only giant unilamellar vesicles (GUVs) as models, we probe the membrane behavior in response to surface topography. Biotin-labeled lipids allow us to tether the vesicles to the surface and to investigate the role of adhesion proteins in the deformation during the vesicle-surface interactions. From confocal microscopy, we image the profile of the vesicle on both planar and topographically patterned substrates. From our model system, we measure the lipid membrane deformation due to the topography, and we compare these findings with the line tension characterization in the literature.@footnote 1@ We model and categorize these responses in our simple system using phase field formulation and compare these findings to cell responses on topographically patterned surfaces. @FootnoteText@ @footnote 1@ Baumgart, Hess and Webb, (2003) Nature 425, 821.

9:00am **BI2-WeM3 Computer Simulation of Water-Mediated Force between Supported Phospholipid Membranes**, *A. Pertsin,* University of Heidelberg, Germany*;* ***M. Grunze,*** University of Maine and University of Heidelberg, Germany

The grand canonical Monte Carlo technique is used to calculate the water-mediated force operating between two supported 1,2-dilauroyl-DL-phosphatidylethanolamine (DLPE) membranes in the short separation range. The intra- and intermolecular interactions in the system are described with a combination of an AMBER-based force field for DLPE and a TIP4P model for water. The long range contributions to the electrostatic interaction energy are treated in the dipole-dipole group-based approximation. The total water mediated force is analyzed in terms its hydration component and the component due to the direct interaction between the membranes. The latter is, in addition, partitioned into the electrostatic, van der Waals, and steric repulsion contributions to give an idea of their relative significance in the water-mediated interaction of the membranes.

9:20am **BI2-WeM4 Phospholipid Bilayers Nanomechanics**, *G. Oncins, S. Garcia-Manyes,* ***F. Sanz,*** University of Barcelona, Spain

Mechanical properties of several phosphocholine supported planar bilayers deposited on mica have been tested in liquid environment by lateral force microscopy (LFM) and force spectroscopy. The presence of these bilayers has been detected topographically using atomic force microscopy (AFM). To test how the presence of NaCl affects the frictional properties of phospholipid bilayers, samples in saline media ranging from 0 M to 0.1 M NaCl were prepared. Changes in the lateral force vs. vertical force curves were recorded as a function of NaCl concentration and related with structural changes induced in the phosphatidylcholine bilayers by the presence of electrolyte ions. Three friction regimes are observed as the vertical force exerted by the tip on the bilayer increases. In order to relate the friction response with the structure of the bilayer, topographic images were recorded simultaneously to friction data. Ions in solution have proved to be able to screen charges present in phosphatidylcholine polar heads, leading to more compact bilayers.@footnote 1@ As a consequence, the vertical force at which the bilayer breaks while performing friction experiments increases with NaCl concentration.@footnote 2@ In addition, images show that low NaCl concentration bilayers recover more easily due to the low cohesion between phospholipid molecules. The vertical mechanical resistance of phosphatidylcholine bilayers has been tested with force curves,@footnote 3@ showing a discontinuity when the bilayer breaks under the pressure exerted by the tip. As expected, the force at which this breakthrough takes place increases with NaCl concentration, pointing out an increase of vertical and lateral mechanical stability induced by ions. @FootnoteText@ @footnote 1@Pandit,S.A.; Bostick, D.; Berkowitz, M. L. Biophys. J., 2003, 84, 3743.@footnote 2@Oncins,G.; Garcia-Manyes, S.; Sanz,F.; (sent 1st revision to Langmuir)@footnote 3@Garcia-Manyes,S.; Oncins,G.; Sanz,F.; (sent 1st revision to Biophys.J.).

9:40am **BI2-WeM5 Fabrication of Nanobiological Materials through Molecular Self-assembly**, ***S. Zhang,*** Massachusetts Institute of Technology
INVITED

Two complementary strategies can be employed in the fabrication of molecular biomaterials. In the 'top-down' approach, biomaterials are generated by stripping down a complex entity into its component parts. This contrasts with the 'bottom-up' approach, in which materials are assembled molecule by molecule and in some cases even atom by atom to produce novel supramolecular architectures. The latter approach is likely to become an integral part of nanomaterials manufacture and requires a deep understanding of individual molecular building blocks, their structures, assembling properties and dynamic behaviors. Two key elements in molecular fabrication are chemical complementarity and structural compatibility, both of which confer the weak and noncovalent interactions that bind building blocks together during self-assembly. Significant advances have been achieved at the interface of biology and materials science, including the fabrication of nanofiber materials for 3-D cell cultures, tissue engineering and regenerative medicine, the peptide detergents for stabilizing, and crystallizing membrane proteins as well as nanocoating molecular for cell organizations. Molecular fabrications of nanobiomateirals have fostered diverse scientific discoveries and technological innovations. Shuguang Zhang made a serendipitous discovery of self-assembling peptides from studying yeast protein, zuotin. He subsequently conceptualized, developed and commercialized diverse self-assembling peptide materials including peptide nanofibers, functional peptide ink, peptide molecular switches and antennae, peptide surfactants/detergents. These self-assembling peptides materials have a broad spectrum of uses, ranging from nanofiber scaffold hydrogel for 3-D tissue cell culture, tissue repair, tissue engineering and regenerative medicine; biochips for direct printing, anchoring and patterning molecules and cells; and peptides for solubilizing, stabilizing and crystallizing membrane proteins. Using systematic and molecular engineering approach, he and his students, postdocs and colleagues opened a new avenue to fabricate novel nanobiological materials from bottom up through molecular self-assembly.

10:20am **BI2-WeM7 1-dimensionally Crosslinked Intra- and Interleaflet Bilayers for Cell Surface Studies**, ***R. Michel***, *M. Halter, G. Sather, E. Naeemi, D.G. Castner,* University of Washington

Although supported lipid bilayers are increasingly used as model systems for biological coatings, they lack the high stability desired for use in ambient or ultrahigh-vacuum environment. Poly(hydroxyethyl methacrylate) (pHEMA) is a hydrogel used in many biomedical applications, most commonly in ophthalmic applications. By tethering lipid bilayers to a pHEMA support, we better mimic the natural environment of the implant. In this work, a twofold approach was employed to stabilize the supported lipid film to the pHEMA. The intra-leaflet stabilization is achieved by cross-linking part of the lipids via the hydrophobic tail using SH-dipalmitoylphosphatidylcholine (DPPC) /acryloyloxy-phosphatidylcholine. To stabilize the leaflet to the surface, dimyristoylphosphatidylethanolamine (DMPE) lipids are mixed with the intra-leaflet crosslinked lipids. The DMPE lipids are crosslinked to the pHEMA substrate via 1.1' carbonyldimidazole (CDI) activation of the pHEMA surface and attachment of the polar head group. Using X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), and fluorescence microscopy, we characterized the purity, composition, and degree of crosslinking of the bilayers. We find that use of these inter- and intra-leaflet crosslinking agents allow us to tailor the fluidity and rigidity of the supported lipid bilayers, which opens up new possibilities for protein incorporation and activity in these lipid bilayers.

10:40am **BI2-WeM8 A Method to Quantify and Evaluate the Efficiency of Nanometer-Sized Lipid Vesicle Modifications**, ***I. Pfeiffer***, Chalmers University of Technology, Sweden*; F. Höök,* Lund University, Sweden

We recently demonstrated a DNA-hybridization-based concept for site-selective and sequence-specific sorting of lipid vesicles on DNA arrays.@footnote1@ By utilizing bivalent cholesterol-based coupling of oligonecleotides to lipid membranes, we showed that the coupling was irreversible in a broad concentration range on planar supported phospholipids bilayers (SPBs) and that exchange between differently modified vesicles in a suspension was sufficiently low to provide efficient sorting. In order to evaluate in further detail the efficiency of this and other lipid vesicle modification protocols, we present in this work a generic method that provides a simple means of quantifying the modification in terms of number of molecules per lipid vesicle. By exposing an SPB to a mixture of cholesterol-modified DNA and lipid vesicles, the amount of free DNA, i.e. DNA not anchored to the lipid vesicles, can be estimated by recording the initial rate of binding to the supported membrane. By comparing the so obtained response with a calibration curve based on the initial rate of binding from suspensions of free DNA, it was demonstrated that the efficiency of the bivalent coupling was 100% in the range of <1 to 35 oligonucleotides per vesicle - thus demonstrating a high stability and efficiency of this particular coupling. The generic value of the method for other types of modifications was demonstrated using cholera toxin binding to GM1 modified lipid vesicles, and given that a solid support can be made inert to colloidal particles, the method holds great promise to be generic for any type of modification scheme. @FootnoteText@ @footnote1@Pfeiffer, I.; Hook, F. Journal of the American Chemical Society 2004, 126, 10224-10225.

11:00am **BI2-WeM9 Mapping Protein Dynamics in Living Cells using Two-Photon Image Correlation Spectroscopy**, ***P. Wiseman***, McGill University, Canada **INVITED**

We will present recent advances in image correlation methods and their application for measurements in living cells. The talk will focus on the development of image correlation spectroscopy (ICS) as an imaging extension of fluorescence correlation spectroscopy (FCS). The ICS technique is ideally suited to measure transport and clustering of fluorescently tagged proteins in cellular membranes where transport is slow and static proteins abound. The image correlation methods are based on the measurement of fluorescence intensity fluctuations as a function of space and time in cells collected as image time series using a laser scanning microscope (either confocal or two-photon). Spatial and temporal variants of the basic ICS method will be introduced and the power of these approaches to measure both aggregation and transport of cell surface proteins will be explained with the aid of computer simulations to demonstrate the measurement detection limits. The use of two-photon microscopy to perform image cross-correlation spectroscopy (ICCS) studies will also be discussed. ICCS allows direct measurement of the interactions of two co-localized proteins labeled with fluorophores having different emission wavelengths even in a high density environment. The transport properties of the co-localized proteins are also measured simultaneously by ICCS. Recent applications of the ICS and ICCS methods for characterizing the transport and clustering of GFP labeled alpha-actinin adhesion proteins in living fibroblasts will be presented. Image correlation studies which demonstrate simultaneous measurement of diffusing and flowing populations of alpha-actinin clusters, and correlated transport between the alpha-5 integrin and intracellular alpha-actinin in CHO fibroblasts at 37C will be shown. We will show spatially resolved vector maps of directed flow of proteins in living cells as measured using our new spatio-temporal ICS method.

11:40am **BI2-WeM11 Microspectroscopic Probing of Intracellular Structures by Observation of Infrared Linear Dichroism in Single Cells in a Micro-Fluidic Cuvette**, ***M. Schmidt***, University of Maine*; M. Rumpler, N. Gierlinger,* Max-Planck-Institut für Kolloid- und Grenzflächenforschung, Germany*; U. Schade,* BESSY GmbH, Germany*; T. Rogge,* Forschungszentrum Karlsruhe GmbH, Germany*; P. Fratzl,* Max-Planck-Institut für Kolloid- und Grenzflächenforschung, Germany*; M. Grunze,* University of Maine and Universität Heidelberg, Germany

Cellular properties and functions are closely related to cell structure. Probing intracellular structures and their dynamic nature is essential for the understanding of the functional characteristics of cells. Infrared (IR) microspectroscopy is an attractive tool for the investigation of biological materials and systems.@footnote 1@ Combining@footnote 2@ this technique with polarization modulation@footnote 3,4@ (PM) and employing synchrotron IR radiation allows us to perform polarization-dependent measurements with high spatial and temporal resolution. Thus, we are able to measure IR linear dichroism (LD) and hence determine preferred molecular orientation of distinct biochemical species in individual cells. Ultimately, observing single living cells in their native environment seems desirable when studying cell structure and function. Therefore, we developed an IR cuvette which facilitates the investigation of individual cells in aqueous solution. This custom-built, demountable and temperature-controllable micro-fluidic cuvette was microfabricated in order to meet the requirements of low pathlength (8 µm) and low volume (1 µL). Our goal is to gain insights into the formation and organization of the cytoskeleton in the context of cell adhesion. Using substrates with well defined surface properties and geometries we seek to control and model cell adhesion. Importantly, IR LD serves as an intrinsic marker for the preferred molecular orientation of the fibrous cytoskeletal proteins. Introduction of external stimuli such as chemicals, mechanical stress and substrate surface variation can be used to study the dynamic response and structural changes inside the cells. @FootnoteText@ @footnote 1@H.Y.N. Holman et al., J. Biomed. Opt. 7, 417 (2002).@footnote 2@Y. Shigematsu et al., Rev. Sci. Instrum. 72, 3927 (2001).@footnote 3@L.A. Nafie and M. Diem, Appl. Spectrosc. 33, 130 (1979).@footnote 4@T. Buffeteau et al., J. Chim. Phys. 90, 1467 (1993).

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