Biomaterial Interfaces

Room: 609 - Session BI+AS+NS-ThA

Surface Analysis and Related Methods for Biological Materials

Moderator: S.L. McArthur, University of Sheffield, UK

2:00pm **BI+AS+NS-ThA1 Creating and Probing Model Biological Membranes**, ***S.G. Boxer***, Stanford University **INVITED**

During the past few years, our lab has developed a wide range of methods for patterning lipid bilayers on solid supports.[1] These 2D fluids are interesting both as a model for biological membranes and as a physical system with unusual properties. Methods have been developed for controlling the composition of patterned membrane corrals by variations on microcontact printing and microfluidics. Charged components can be moved around within these fluid surfaces by a form of 2D electrophoresis. The planar geometry of supported bilayer systems is ideal for high resolution imaging methods. The lateral (x-y) composition of membranes can be analyzed by high spatial resolution secondary ion mass spectrometry (SIMS) using the NanoSIMS 50 (Cameca) at the Livermore National Laboratory. Results will be described for simple membrane compositions[2] and phase separated domains[3] suggesting the potential of this method for the analysis of membrane organization in complex membranes. Extensions of this approach to more complex systems including membrane-associated proteins will be described. If time permits, a complimentary optical imaging method offering sub-nm resolution in the z-direction (perpendicular to the membrane surface) will be described in the context of imaging conformational changes in membrane proteins.

[1]J. T. Groves and S. G. Boxer, Accounts of Chemical Research, 35, 149-157 (2002).
[2]C. Galli Marxer, M. L. Kraft, P. K. Weber, I. D. Hutcheon and S. G. Boxer, Biophysical Journal, 88, 2965-2975 (2005).
[3]M. L. Kraft, P. K. Weber, M. L. Longo, I. D. Hutcheon, S. G. Boxer, Science, 313, 1948-1951 (2006).

2:40pm **BI+AS+NS-ThA3 Activation Thermodynamics for Phospholipid Flip-Flop in Planar Supported Lipid Bilayers Measured by Sum-Frequency Vibrational Spectroscopy**, ***T.C. Anglin***, *H. Li, J.C. Conboy*, University of Utah

Basic transition state theory is used to describe the activation thermodynamics for phospholipid flip-flop in planar supported lipid bilayers prepared by the Langmuir-Blodgett / Langmuir Schaeffer method. Kinetics of flip-flop are determined as a function of varying temperature and lateral surface pressure for model bilayers of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) using sum-frequency vibrational spectroscopy (SFVS). The temperature and pressure dependence of the kinetics of DSPC flip-fop provide a complete description of the activation thermodynamics for flip-flop in the gel state, including free energy of activation, area of activation, and entropy of activation. This is the first description of phospholipid flip-flop according to basic transition state theory with explicit treatment of the free-energy dependence of the process and determination of the entropic contribution to the transition state.

3:00pm **BI+AS+NS-ThA4 Observation of Electrical Characteristics at Cells Membrane using by Electrostatic Force Microscopy**, ***Y.J. Kim***, Myongji University, Korea, *H.D. Kim,* Seoul National University, Korea, *Y.S. Kim,* Myongji University, Korea, *K.H. Lee,* Seoul National University, Korea, *C.J. Kang*, Myongji University, Korea

Recent advances in atomic force microscopy (AFM) made it possible to investigate the biological materials in a single molecule level. Moreover, the AFM has been used to measure the fine structure of individual live cell even under physiological liquid. The images of cells measured show finer structure of cell boundary compared with those of SEM after fixation. Since electrostatic force microscopy (EFM) using conducting cantilever to AFM allows us to observe the electrical properties of the surface, it is also used to study the various properties of the cell membrane. Reportedly, it is known that protein expression depends on the cell kinds and shows the non uniform distributions, which causes the electrical potential difference on the cell surface in the local area. Thus mapping the electrical potential of a cell using EFM and comparing it with that of reference group, we are able to extract the information to differentiate the cells. In this work, we have identified the breast cancer cells (MCF7) and normal breast epithelial cells (MCF10A) derived from the same origin by fractal dimension analysis using AFM and the electrical properties of the cell membrane measured from the EFM will be also discussed. The results show that AFM imaging with EFM measurement might be feasible methods for analyzing surface structures of living cells with high resolution, and it could provide new insights into cell surface structure.

3:40pm **BI+AS+NS-ThA6 Two and Three Dimensional Analysis of C. Albicans Biofilms with Cluster SIMS**, ***B.J. Tyler***, *S. Rangarajan*, University of Utah, *J. Moeller, H.F. Arlinghaus*, University of Muenster, Germany

The high tolerance of microbial biofilms to important antimicrobial agents creates an import problem for treatment of infections associated with implanted medical devices. Several important hypotheses for this drug resistance involve mass transport limitation within the biofilms. We have been using 2_D and 3_D ToF-SIMS analysis to investigate transport of drugs and nutrients through C. albicans biofilms. Analysis has been performed using a novel ToF-SIMS system which incorporates a cryo-sectioning chamber and precise temperature control during analysis. This instrument has allowed us to map the distribution of key nutrients and drugs with in the biofilm as well as to identify viable vs. nonviable cells. Through these studies, we have determined that the multiple cellular layers and extracellular polymers are not the most important mass transport barrier. Common drugs are able to permeate to the bottom of the biofilm within 5 minutes but are still unable to penetrate the cell wall of persister cells found at the base of the biofilm. Current work is focusing on cell wall and membrane changes within a subpopulation of the biofilm cells which apparently limits transport of antimicrobials into the cells. Multivariate statistical techniques have been crucial for the analysis of these samples and discussion of the relevant statistical methods employed will be presented.

4:00pm **BI+AS+NS-ThA7 XPS, ToF-SIMS and NEXAFS Investigation of Peptide Adsorption onto SAMs**, ***J.S. Apte***, *L.J. Gamble, D.G. Castner*, University of Washington

The interactions between proteins and surfaces are critical to the success or failure of implants in the body. When adsorbed onto a synthetic surface, proteins often denature which can trigger the foreign-body response. It is therefore essential to develop methods to examine these interfacial phenomena. This work uses X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS) and near-edge X-ray absorption spectroscopy (NEXAFS) to characterize the structure of α-helix and β-sheet peptides adsorbed onto self-assembled monolayers (SAMs). The α-helix peptide is a 14-mer made up of lysine (K) and leucine (L) residues with a hydrophobic periodicity of 3.5. The β-sheet peptide is a 15-mer also made up of L and K residues with a hydrophobic periodicity of 2. A β-sheet peptide with the same structure but with valine (V) substituted for L was also studied since V has a higher tendency than L to form β-sheet structures. All peptides have the hydrophobic side-chains on one side of the peptide and the hydrophilic on the other. The SAMs studied were thiols on gold containing the ω-groups $-CH_3$, -OH, -COOH, $-NH_3^+$ and $-CF_3$. XPS nitrogen atomic percent was used to measure adsorption isotherms for the peptides. The α-helix peptide forms a monolayer (8.2% N) on the COOH-terminated SAM at an adsorption concentration 50 times lower than on the CH_3-terminated SAM (0.01 mg/mL compared to 0.5). The surface coverage on the CH_3 SAMs appeared to be patchier compared to the COOH SAMs, since they had standard deviations of 2-3% N. Atomic force microscopy images of the adsorbed peptides were generated to examine this patchiness. Also, the adsorption process depended on buffer salt concentration. Little peptide adsorption was detectable on the methyl SAMs when adsorbed from a 0.1x buffer. ToF-SIMS was used to investigate the ratio of K to L characteristic mass fragments at 84 and 86 m/z, respectively. The 84/86 (K/L) ratio on CH_3 SAMs (1.2) was the same, within experimental error, as the ratio on COOH SAMs (1.1). Polarization dependent NEXAFS experiments at the nitrogen K-edge indicated the β-sheet was lying down on the SAM surfaces. The α-helical peptide exhibited significantly less polarization dependence than the β-sheet peptide, probably due to the different structure of the backbone amide groups in the α-helical peptide.

4:20pm **BI+AS+NS-ThA8 Isolation and Detachment of Small Cell Populations from a Thermoresponsive Polymer**, ***H.E. Canavan***, *K. Gallagher-Gonzales, J.A. Reed*, University of New Mexico

Poly(N-isopropyl acrylamide) (pNIPAM) has proven to be an efficient and non-destructive means of detaching intact sheets of mammalian cells. In addition, cell sheets detached from pNIPAM maintain their association with the extracellular matrix (ECM) during and following detachment from a coated surface, enabling their use in tissue engineering. To date, the majority of those studying cellular interactions with pNIPAM have focused on harvesting large domains of cells for such tissue engineering applications. However, there are many other applications for which the non-

destructive release of smaller populations, or even isolated cells, is desirable. For example, isolated cells are required to ascertain the extent of transmembrane protein receptor upregulation when assaying the efficacy of cancer therapeutics on cell populations via flow cytometry (FC). In this work, arrays of thermoresponsive domains were fabricated to isolate defined populations of cells using a variety of techniques. The surface chemistry, thermoresponse, and topography of the films generated were verified via X-ray photoelectron spectroscopy (XPS), contact angle measurements, and atomic force microscopy (AFM), respectively, and compared to controls. The cell releasing properties of the films were characterized by incubating baby hamster kidney (BHK) and bovine aortic endothelial cells (BAECs). The behavior of the cells from isolated cells and small cell populations were characterized and compared to large cell population controls.

4:40pm **BI+AS+NS-ThA9 Surface Characterization of Ordered Nanopatterns made from Self-Assembly of Mixed Nanoparticles**, *S. Pillai, G. Singh,* The University of Aarhus, Denmark, *C. Blomfield, A. Roberts,* Kratos Analytical Ltd, UK, *R.L. Meyer,* ***P. Kingshott***, The University of Aarhus, Denmark

Controlled patterning of surfaces with different chemistries and structures at nanoscale length scales is highly desirable for understanding the fundamental mechanisms of protein and cell interactions with biomaterials. The use of nanoparticles (NPs) to pattern surfaces by colloidal lithography or templating using one type of NP is well known for applications such as biosensors,[1] biomaterials[2] and tissue engineering.[3] We demonstrate that by using mixed nanoparticles unique highly-ordered patterns can be obtained by simple self-assembly from buffer onto hydrophobic surfaces from both concentrated and dilute two-component NP suspensions.[4] The new method uses suspensions of poly(styrene) (PS) NPs of different size (d = 500 to 60nm) with different NP ratios and volume fractions. The ordering is independent of the NP surface chemistry (sulfated, carboxylated, or aminated PS) or zeta potential and occurs over a broad pH range (4-10). The method is demonstrated for two types of hydrophobic surfaces, a commercial adhesive carbon tape and octadecyltrichlorosilane (OTS)-modified glass where patterns of hexagonally packed large particles are inter-dispersed with smaller particles. However, very poor ordering is achieved using gold, mica and HOPG (highly oriented pyrolytic graphite) as substrates. The morphologies of the nanoparticle assemblies are characterised extensively by SEM and AFM. In addition, XPS and ToF-SIMS are used to characterise the surface chemistry of the NP surfaces, which also provides knowledge of the mechanisms of NP assembly by showing that the ordering is most likely associated with charge screening by buffer salts resulting in an entropically driven assembly process. These preliminary results indicate that use of two component NP assemblies opens up the possibilities of decorating surfaces with well-defined chemical nanopatterns capable of selective attachment of different proteins and/or protein resistant molecules.

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[3]S.N. Bhatia , U.J. Balis, M.L. Yarmush, M. Toner, FASEB J. 1999, 14, 1883.
[4]R. Mukhopadhyay, O. Al-Hanbali, S. Pillai, A. Gry Hemmersam, R.L. Meyer, C.A. Hunter, K.J. Rutt, F. Besenbacher, S.M. Moghimi, P. Kingshott, submitted.

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