In Situ Microscopy and Spectroscopy Topical Conference
Room: Acoma - Session IS+BI+AS-WeA

In Situ Microscopy/Spectroscopy – Biological Interfaces
Moderator: M. Grunze, University of Heidelberg, Germany

2:00pm **IS+BI+AS-WeA1 Adsorption and Phase Transition of Liposomes via Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy**, ***M.R. Hernandez,*** *T.C. Ng, E.N. Towns, B.C. Walsh, D.P. Land*, University of California at Davis

Liposomes are becoming increasingly prevalent as an important part of drug delivery systems in modern medicine, however a better understanding of the physical characteristics is needed. In this study we present our results on the stability and adsorption of liposomes formulated from dipalmitoylphosphatidylcholine (DPPC) via attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. The phase transition temperature of liposome formulations of pure DPPC, DPPC and cholesterol, and DPPC, cholesterol, and 1,2-Distearoyl-phosphatidylethanolamine-methyl-polyethyleneglycol -2000 (DSPE-mPEG2000) is determined using a temperature dependant study (25°C to 50°C) and been found to occur abruptly around 41°C for pure DPPC liposomes and exhibit gradual temperature changes from 35°C to 43°C for the other two liposome formulations. We have studied the adsorption characteristics of different formulations of liposomes with both hydrophobic and hydrophilic surfaces created by different self-assembled monolayers, and will present a new method for studying an in vitro way of studying the adsorption of different formulations of liposomes onto a surface of immobilized proteins. Knowing the stability of liposomes and liposome-protein adsorption characteristics allows for a better understanding of their use and design of future formulations in drug delivery systems.

2:20pm **IS+BI+AS-WeA2 Synchrotron Based Infrared Imaging at the Diffraction Limit**, *J. Nasse,* University of Wisconsin-Milwaukee*, C. Gohr, A. Rosenthal,* Medical College of Wisconsin*,* ***C. Hirschmugl***, University of Wisconsin-Milwaukee

A new mid-infrared beamline (IRENI) extracting a large horizontal swath of radiation (320 hor. x 25 vert. $mrads^2$) to homogeneously illuminate a commercial IR microscope equipped with an infrared Focal Plane Array (FPA) detector has recently been commissioned at the Synchrotron Radiation Center in Stoughton, WI. This new facility provides the opportunity to obtain chemical images with diffraction-limited resolution, for all wavelengths in the mid-IR concurrently, in minutes. The design of this facility and an initial application will be presented.

IRENI combines a bright IR synchrotron source to an FTIR microscope with a multi-element detector for wide-field imaging as opposed to the common dual-aperture geometry with raster scanning that is available at most synchrotron IR beamlines. The swath of radiation from the SRC is extracted as 12 beams and recombined into a 3 x 4 bundle of beams that is refocused onto a sample plane of an infrared microscope illuminating 40 x 60 $micron^2$ sample area. The sampled spatial resolution is defined by both the magnification after the sample and the FPA pixel size. Here, a 74x Schwarzschild objective achieves effective geometric pixel sizes of 0.54 x 0.54 $micron^2$, which is approximately $\lambda/4$ for even the shortest wavelength of 2 µm. This spatial oversampling provides adequate information to obtain concurrent, diffraction-limited images across the entire spectral range. In addition, the spectral quality is excellent, since the high density, stable, broadband flux from the synchrotron achieves high quality spectra for 0.54 x 0.54 $micron^2$/pixel using similar measuring times as table-top instruments that image 5.5 x 5.5 $micron^2$/pixel.

The presence of calcium-containing crystals, including calcium pyrophosphate dihydrate (CPPD) and hydroxyapatite-like basic calcium phosphate (BCP), in synovial fluids plays a major role in cartilage degeneration in osteoarthritis. Models of calcium crystal formation tend to produce small, sparse crystals embedded in debris enriched in proteins, lipids, and carbohydrates, which interfere with many identification techniques. Synchrotron FTIR imaging circumvents difficulties in identifying these crystals and also allows for characterization of the surrounding matrix. We present results from well-characterized models of calcium crystal formation that demonstrate our ability to both identify crystals *in vitro* and characterize the matrix surrounding these crystals.

This work has been done with support from an NSF Major Research Instrumentation grant (DMR-0619759) and the Synchrotron Radiation Center, which is also supported by NSF (DMR-0537588).

2:40pm **IS+BI+AS-WeA3 Proteins and Lipids at Liquid/Solid Interfaces: *In situ* Studies by Neutron Reflectometry and Infrared Spectroscopy**, *M. Strobl, M. Kreuzer,* University of Heidelberg, Germany, *M. Reinhardt, R. Steitz,* Helmholtz Zentrum Berlin, Germany, *M. Grunze,* ***R. Dahint***, University of Heidelberg, Germany **INVITED**

Proteins and lipids at liquid/solid interfaces are of crucial importance in the design of biofunctional interfaces. For example, adsorbed protein layers determine the biocompatibility of implants and may control bacterial adhesion. Upon surface contact, proteins commonly undergo structural changes, which will alter their activity and biological function. In combination with lipids, proteins are valuable model systems to mimic cell membrane function. Thus, in order to improve our understanding of biofunctional interfaces, a strong need exists to develop surface analytical tools, which facilitate *in situ* characterization on a molecular level.

Due to its *in situ* capability, non-destructive character and the short wavelength of neutron beams, neutron reflectometry offers a very attractive approach to the analysis of layer structures on the nanometer scale. It provides detailed information on the amount of adsorbed species as well as on the thickness, density and hydration of the absorbate. In combination with surface sensitive infrared spectroscopy (ATR-FTIR), additional information is obtained on specific molecular groups of the adsorbate as well as on molecule conformation.

We will report on the set-up of a new time-of-flight neutron reflectometer at the Helmholtz Center Berlin, which is especially adapted to biological samples and, for the first time, facilitates simultaneous *in situ* ATR-FTIR characterization. Dedicated sample environments have been developed to study biological films as a function of applied pressure, shearing forces and temperature. As a potential application, we discuss the phase behavior and stability of immobilized oligolamellar lipid bilayer films under load and shear, which are important in bio-lubrication and the search for advanced implant materials, such as artificial joints. A second example will focus on the impact of surface chemistry and structure on the activity of immobilized proteins.

4:00pm **IS+BI+AS-WeA7 Biological Imaging with Coherent X-rays: The Lens-less Approach to High Resolution**, ***A. Beerlink***, Universität Göttingen, Germany **INVITED**

Understanding molecular functions in complex environments such as biological cells or novel composite materials are a prerequisite for the advancement of nano and biomedical sciences. They require a combination of high spatial resolution, quantitative contrast and

full compatibility with environmental conditions, such as aequous media. To this end, the potential of x-ray imaging is not yet fully developed, but currently undergoes rapid progress. While classical x-ray microscopy based on Fresnel zone plates has matured and provides useful structural information in a growing range of applications, this technique is severely limited by the nanostructuring process of the lenses. In

recent years, novel lens-less approaches for imaging have emerged, where the object functions are reconstructed from the measured intensities

either in the far-field regime, or under near-field conditions (propagation imaging). We present experiments using x-ray quasi point sources to illuminate the sample in combination with digital recording of the resulting diffraction patterns. One focus is the applicability towards biological samples, for which the imaging properties of the different coherent microscopy approaches will be compared. In this context, recent results obtained with ultrabright femtosecond pulses provided by the free electron laser FLASH will be presented and accessible information complementary to synchrotron imaging will be discussed.

4:40pm **IS+BI+AS-WeA9 Dielectric Constant and Polarization of Biomolecules Determined by Torsional Resonance Nanoimpedance Microscopy**, ***K. Kathan-Galipeau***, *S.U. Nanayakkara, P.A. O'Brien, B.M. Discher, D.A. Bonnell*, University of Pennsylvania

We have developed a new technique, torsional resonance nanoimpedance microscopy (TR-NIM), that allows for the measurement of frequency-dependent local transport properties on soft materials. AFM measurements at torsional resonances provide a key advantage: the ability to achieve low-force scanning while maintaining the tip in the near-field. As a result, it is possible to measure impedance between the tip and sample without damaging the sample.

This technique has been used to determine the resistance, capacitance, and dielectric properties of a novel class of biomolecules. These redox active molecules, known as maquettes, consist of dimers and tetramers of alpha helix polypeptides and provide a convenient functional alternative to natural

proteins. Maquettes are capable of binding a range of cofactors; this study examines the properties of iron and zinc porphyrins. Maquettes serve as a benchmark for integrating electronics with biologically inspired materials that possess unique characteristics, such as electron-transfer capability, the possibility of gating redox activity, optoelectronic functionality, and nanometer size.

In order to determine the behavior of these functional biomolecules on electrodes, PDMS stamping was used to create stripes that alternate between maquettes and bare graphite. Stamping also allowed us to control the assembly of the redox-active maquettes from multilayers to horizontally oriented monolayers (maquettes laying down) and vertically oriented monolayers (maquettes standing up) by varying the stamping time and the concentration of the maquettes in organic solvents.

Interestingly, we observe that the resistance decreases with increased height of maquettes, which is explained in terms of the configurations of the molecules on the electrode. The dependence of local impedance on exposure to optical radiation revealed an increase in capacitance and decrease in resistance when the maquettes are exposed to 425 nm light. This is true for both zinc and iron porphyrin cofactors. We attribute the decrease in resistance to photoactivated current. The increase in capacitance is due to an increase in the polarizibility of the maquettes.

5:00pm **IS+BI+AS-WeA10 Dynamic Observation of Phospholipid Model Cell Membranes and Particles by STM and Vibrational Spectroscopy**, ***T. Yamada***, RIKEN, Japan, *S. Matsunaga,* The University of Tokyo, Japan, *T. Kobayashi,* RIKEN, Japan, *M. Kawai*, The University of Tokyo, Japan

Scanning tunneling microscopy (STM) and other surface-scientific techniques can be utilized to explore the microscopic dynamics of biological molecules in the context that the techniques are applicable for solid surfaces immersed in aqueous solutions. We devised STM and vibrational spectroscopies to make usable for molecular monolayers at solid-liquid interface. We attempted to observe phospholipid layers formed on octanethiol-terminated gold (111) single-crystalline substrates placed in aqueous buffer solutions (in situ STM). By in situ STM we could observe dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), a relatively short kind of lipid, forming a fluidic monolayer. A crystalline phase of this monolayer was observed by applying an electrode potential compatible with the membrane potentials of real cells. Furthermore, mixed lipid layers have been examined by STM [1]. We found some nanometer-scale raft structures (phase-separated domains), which are functionally characteristic for real cell membranes. We also studied phospholipid particles suspended in buffer solutions. Suspensions were prepared from a phosphcholine (PC) and an ethanolamine (PE), consisting of nanometer-scale phospholipid particles with narrow size distribution. In situ STM revealed particles with a diameter ~ 10 nm (named “minimal lipid particles (MLP)”), forming a monolayer along the Au(111). It is known that some categories of antibiotics selectively attack lipids contained in germ cell membranes and disintegrate the whole cells. We chose “duramycin”, a 19-residued peptide antibiotic, which specifically binds PE. When the total concentration of phospholipid was controlled between 100 μM and 500 μM, a layer of MLP was discerned. During STM scanning, 7 μM of duramycin solution was added into the suspension, and the PC+PE MLP became fragile and seemed to be scratched by the tip, ending up with a widespread multilayer. This sort of highly leveraged effect of duramycin is characteristic in the action of antibiotics [2]. These works demonstrated the advantage of STM in monitoring the live nanometer-scale reactions of biological entities, which have not been recognized experimentally so far. We expect more application of STM in physiological investigation in cell biology.

[1] S. Matsunaga et al., *Electrochem. Commn*. **9** (2007) 645.

[2] S. Matsunaga et al., *Langmuir* **25** (2009) 8200.

5:20pm **IS+BI+AS-WeA11 Rapid In-Situ Assessment for Microbes on Simultaneously Prepared Plate with Substrate and Zirconium Based Thin Film Metallic Glasses (TFMGs)**, ***P.T. Chiang***, I-Shou Univ./Fooyin Univ. Hospital, Taiwan, Republic of China, *G.J. Chen, H.H. Liu, Y.H. Shih,* I-Shou Univ., Taiwan, Republic of China, *J.P. Chu,* National Taiwan Univ. of Science and Technology, Taiwan, Republic of China, *J.S.C. Jang*, National Central Univ., Taiwan, Republic of China

ZrAlNiCuSi TFMGs could modify the stainless steel’s surface with high hardness, scratch-adhesion capabilities. Zr-based TFMGs’ smooth surface could decrease and prolong the lag phase of microbes’ growth for at least 24 hours.

The actual numbers of pathogenic bacteria might be underestimated by conventional methods due to sublethal injury, malnutrient’s and other physiological factors which reduce bacterial viability. Moreover, these methods would limit the real-time quantitative detection and easily cause contaminations with bias.

Rapid comparisons in the same culture condition are obtained on a simultaneously prepared plate with substrate and Zr based TFMGs. By utilization of GFP plasmid (pGLO) into HB101 with 10mM arabinose induction, we could measure the intensity of green fluorescence by LAS-3000 fluorescent detector to setup the real-time monitor system for observation of bacterial growth on TFMGs’ surface.

This integrated method was time-saving, cost-effective and simple. The serially rapid in situ monitor of the microbial growth will emerge as a novel tool to realize the TFMGs or other materials’ antimicrobial properties.

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