Wednesday Afternoon, October 22, 2008

Biomaterial Interfaces
Room: 202 - Session BI-WeA

Quantitative Analysis of Biointerfaces
Moderator: D.G. Castner, University of Washington NESAC/BIO

1:40pm **BI-WeA1 'Structure' of Water: Myth or Reality?**, ***M. Grunze***, University of Heidelberg, Germany **INVITED**

The "structure" of water at interfaces is a reoccurring interpretation of experimental data or computer simulations employing simplified models for liquid water. Oriented binding of water molecules to a hydrogen bridge bond donor or acceptor group, as observed e.g. in vibrational spectroscopies, does not imply translational symmetry or "structure", and the orientational order parameter decays typically with the second hydration shell. Whereas the practical significance of surface energy and hence wetability of organic surfaces for adhesion, adhesion failure and biomolecule interaction is obvious, the molecular basis of the hydrophobic and hydrophilic properties of organic surfaces is poorly understood. This is due to the lack of suitable experimental tools for detailed spectroscopy studies of organic interfaces in liquid environments and of molecular detailed theoretical descriptions of hydrophobic/hydrophilic interactions. In this talk I will briefly discuss the limits and potentials of experimental approaches to interfacial water (Neutron Reflectivity, Sum Frequency Generation Spectroscopy, and Terahertz Spectroscopy) to develop a molecular understanding of the properties of water in organic interphases of different surface energy; and time permitting get back to a long standing controversy, the "inert" surface problem, which is a good example for demonstrating the complexity of interfacial water properties.

2:20pm **BI-WeA3 A Novel Approach to XPS Characterisation of 'Click' Surface Chemistry, More Information Less Damage**, ***T.S. Nunney***, *R.G. White*, Thermo Fisher Scientific, UK, *N.B. Larsen, T.S. Hansen, A.E. Daugaard, S. Hvilsted*, Technical University of Denmark

'Click' chemistry is increasingly used for chemical surface engineering of polymer devices to be used in biological and medical applications. Advantages of 'click' chemistry include mild reaction conditions, i.e. aqueous environment at room temperature, and high chemical specificity of the coupling. We recently demonstrated surface engineering of ultrathin electrically conductive polymer films by 'clicking' organic functional units that control wettability, protein adhesion, or fluorescence, all functions of major relevance to biomedical applications. The most commonly used click reaction is based on the coupling of organic azides to alkynes. This is also the basis of our recently reported functional monomer, azide modified 3,4-ethylenedioxythiophene, for conducting polymer films (PEDOT-N3) reactive towards alkyne functionalized molecular species. The ability of X-ray photoelectron spectroscopy (XPS) to provide quantitative chemical state information makes it ideal for the investigation of the resultant clicked surface chemistry. In the example above, differences in the XPS binding energy for the azide and triazole nitrogens serve as a useful method to determine if the click reaction has completed successfully. It is known, however, that degradation of the azide chemistry during XPS measurement process can significantly obscure the result. In this paper we will discuss methods for minimisation of the measurement-induced chemical degradation. These methods rely on a number of hardware and software features which have recently become available on modern XPS instrumentation. The methods described require the layers to be uniform so that the data can be collected as a map, thus reducing the X-ray and electron flux density during the measurement. Deconvolution routines will be shown to facilitate the rapid chemical state mapping of patterned variants of these surfaces.

2:40pm **BI-WeA4 A New QCM-D and Reflectometry Instrument - Applications to Supported Lipid Structures and their Interactions**, ***M. Edvardsson***, *S. Svedhem*, Chalmers University of Technology, Sweden, *G. Wang*, Chalmers University of Technology and Q-Sense AB, Sweden, *R. Richter*, CIC biomaGUNE, Spain, *M. Rodahl*, Q-Sense AB, Sweden, *B. Kasemo*, Chalmers University of Technology, Sweden

In the past decade, the Quartz Crystal Microbalance with Dissipation monitoring technique (QCM-D) has emerged as a powerful biosensor technique.[1] A key feature of the technique is that the shift of the resonant frequency, Δf, obtained upon adsorption of mass on the QCM-D sensor surface includes both the actual mass and solvent (e.g. water) associated with it. For a rigid film containing no water (low dissipation shifts, ΔD), the frequency shift, Δf, can be considered proportional to the mass of the film. For viscoelastic films containing water (high dissipation shifts), however, it is difficult to determine how much of the frequency shift results from the actual adsorbed mass and how much is a contribution from entrapped or associated water. In some applications, the signal enhancement that is obtained through the associated liquid, makes the QCM-D technique unique with respect to the added information that is gained compared to, for example, optical techniques. In particular, spontaneous fusion of lipid vesicles onto solid supports have been studied extensively using the QCM-D technique,[2] and unique new information has been obtained. However, for a full picture one would, for such complex viscoelastic films, ideally combine the QCM-D technique with a technique that allows separation of the adsorbed (non-hydrated or "dry") mass and the associated liquid (wet mass). This presentation demonstrates applications of a recently developed instrument, combining, on the same sensor surface, the QCM-D technique and optical reflectometry [Wang et al., submitted to Rev. Sci Instr,], for surface based analysis of biomolecular and polymer adlayers. The combination instrument makes it possible to do simultaneous, time-resolved measurements of hydrated and non-hydrated mass and viscoelastic properties of films and molecular adlayers formed on the surface. The experimental setup is described, and the value of this combination of techniques is demonstrated via applications on model systems that involve supported lipid structures of various degree of hydration; ranging from systems of low water content, e.g., bilayers, to those of high water content, such as surface-attached vesicles and bilayers with a highly hydrated peptide coupled to it.

[1]Cooper, M. A.; Singleton, V. T. J. Mol. Rec. 2007, 20, 154-184.
[2]Richter, R. P.; Bérat, R.; Brisson, A. Langmuir 2006, 22, 3497-3505.

3:00pm **BI-WeA5 Calculation of Adsorption Free Energy for Peptide-Surface Interactions using Molecular Dynamics Simulation Methods**, ***N. Vellore***, *S.J. Stuart*, Clemson University, *B.R. Brooks*, National Institutes of Health, *R.A. Latour*, Clemson University

While it is well understood that protein-surface interactions are of fundamental importance for understanding cell-surface interactions, very little is understood at this time regarding the molecular level events that control protein adsorption behavior. Molecular dynamics simulations methods have enormous potential for development as a tool to help understand and predict protein adsorption behavior. These methods, however, must first be developed and validated for this specific application. One of the most important areas for development is the assessment and validation of force field parameters that will enable the competition between amino acid residues of a peptide or protein and molecules of the solvent (i.e, water and salt ions) for the functional groups presented by a surface. One of the fundamental driving forces that control these types of interactions is the free energy of adsorption. We have therefore developed a method of accurately calculating the adsorption free energy of peptide-surface interactions using molecular dynamics simulations with an advanced sampling algorithm called biased replica-exchange molecular dynamics (biased-REMD). Simulations are performed with the CHARMM force field and simulation package using explicitly represented solvent (150 mM Na+/Cl- in TIP3P water) with periodic boundary conditions. A host-guest peptide model is used for these simulations in the form of TGTG-X-GTGT, where the T (threonine) and G (glycine) flanking sequences are the host residues and X represents a variable guest residue. Alkanethiol self-assembled monolayers (SAMs) with a broad range of polymer-like functionalities are being used as the adsorbent surfaces. The results of these simulations are being compared with complementary experimental studies using these same peptide-SAM systems in order to evaluate the accuracy of the force field, and to provide a basis for force field parameter modification for the development of a validated force field parameter set for the accurate representation of peptide-surface interactions. Once developed, these methods will be able to be applied to accurately simulate protein-surface interactions, thus providing a valuable resource to investigate protein-surface interactions at the molecular level.

4:00pm **BI-WeA8 Nonlinear Light Scattering: Bridging the Gap between Surface Science & Soft Matter**, ***S. Roke***, Max-Planck Institute for Metals Research, Germany **INVITED**

Interfaces play a key role in many processes. They play a regulating role in transport and structural phenomena in biological cells, they can determine the chemistry and (phase) behavior of colloidal systems, they are important for the mechanical properties of (amorphous) solids and they determine the electrical properties of micro- and nano-electronics. When the size of materials decreases down to the level of micro- or nano-structures, the relative interfacial area increases. For small systems it is well-known that the interfacial region becomes a dominating factor in determining the physical and chemical properties of a material. Thermodynamically, on a

macroscopic level, the effect of an interface region is well understood. On a molecular level, however, it is not. In this presentation I will introduce vibrational sum frequency scattering as a novel method to investigate particle and domain interfaces,[1] highlight new possibilities that become available and show some of the latest developments. These include: the possibility of investigating molecular surface effects in colloidal phase transitions,[2,3] how to extract molecular properties[4] and the possibility of observing embedded domain structures in polymorph materials.[5]

[1] - S. Roke, W. G. Roeterdink, J. E. G. J. Wijnhoven, A. V. Petukhov, A. W. Kleyn and M. Bonn, Phys. Rev. Lett., 91 (2003), 258302-1.

[2] - S. Roke, J. Buitenhuis, M. Bonn and A. Van Blaaderen, J. Phys.: Condens. Matter., 17 (2005), S3469-S3475.

[3] - S. Roke, J. Buitenhuis, A. van Blaaderen and M. Bonn, Proc. Nat. Acad. Sci, 103 (2006), 13310-13314.

[4] - A. G. F. de Beer and S. Roke, Phys. Rev. B, 75 (2007), 245438-1-8.

[5] - A. G. F. de Beer, H. B. de Aguiar, J. F. W. Nijsen, A. B. Sugiharto and S. Roke, submitted.

4:40pm **BI-WeA10 Characterization of DNA Monolayers on Gold using Sum Frequency Generation Spectroscopy**, ***C.L. Howell***, *M. Grunze, P. Koelsch*, University of Heidelberg, Germany

We investigated a series of model monolayers of single stranded DNA (ssDNA) on gold using broadband femtosecond Sum Frequency Generation (SFG) Spectroscopy. SFG processes, involving a non-linear resonant responses produced by exciting vibrational modes of molecular bonds using overlapping IR and visible beams, are inherently interface-specific. The surface specificity of SFG, combined with polarization dependence, allows for the investigation of the ordering and orientation of molecules at surfaces in air and through bulk solutions. However, due to the difficulties associated with operating an SFG system in biological relevant spectral regions (such as the amide I and fingerprint), combined with the difficulties of interpreting vibrational spectra from complex biomacromolecules, there are few published examples of characterization of DNA films using SFG. Our goal is to create high quality vibrational SFG spectra of model monolayers of DNA on gold and to compare these spectra to results obtained from complementary surface spectroscopies that have been applied to DNA monolayers, such as XPS, FTIR, and NEXAFS. Preliminary results in the C-H stretching region show distinctive changes in the locations and relative intensities of peaks for a film of thiol-modified 5-mers of thymine (T5-SH) compared to thiol modified 25-mers (T25-SH) and unmodified thymine 5-mers (T5). Preliminary SFG spectra of ssDNA films in the Amide I region revealed changes in the locations and intensities of the major peaks for the T5 film compared to a film of unmodified adenine 5-mers, as well as compared to T5-SH and T25-SH films. Refinement and validation of SFG Spectroscopy as a tool for the characterization of DNA monolayers on gold could provide another method for examining the structure of these films, and potentially serve as a bridge for comparing these systems ex situ and in situ.

5:00pm **BI-WeA11 Avoiding Parasitic Reactions Due to Interconnect Dead Volume and Non-Specific Binding in Microfluidics**, *X. Luo,* ***D.L. Berlin***, *W.E. Bentley, G.F. Payne, R. Ghodssi, G.W. Rubloff*, University of Maryland

Biological microelectromechanical systems (bioMEMS) provide an attractive approach to understanding and modifying enzymatic pathways by separating and interrogating individual reaction steps at localized sites in a microfluidic network. We have previously shown that electrodeposited chitosan enables immobilization of an enzyme at a specific site while maintaining its catalytic activity. While promising as a methodology to replicate metabolic pathways and search for inhibitors as drug candidates, these investigations also revealed unintended (or parasitic) effects, including products generated by the enzyme either (1) in the homogeneous phase (in the liquid), or (2) nonspecifically bound to microchannel surfaces. Here we report on bioMEMS designs that significantly suppress these parasitic effects. To reduce homogeneous reactions we have developed a new packaging and assembly strategy that eliminates fluid reservoirs that are commonly used for fluidic interconnects with external tubing. To suppress reactions by nonspecifically bound enzyme on microchannel walls we have implemented a cross-flow microfluidic network design so that enzyme flow for assembly and substrate/product flow for reaction share only the region where the enzyme is immobilized at the intended reaction site. Our results show that the signal-to-background ratio of sequential enzymatic reactions increases from 0.72 to 1.28 by eliminating the packaging reservoirs, and increases to 2.43 by separating the flow direction of enzymatic reaction from that of enzyme assembly. These techniques can be easily applied to versatile microfluidic devices to minimize parasitic reactions in sequential biochemical reactions.

5:20pm **BI-WeA12 Probing Orientation and Conformation of α-Helix and β-Sheet Model Peptides on Self-Assembled Monolayers with SFG and NEXAFS Spectroscopy**, ***T. Weidner***, *J. Apte, L.J. Gamble, D.G. Castner*, University of Washington

Understanding the interaction of proteins and peptides with engineered surfaces from first principles is essential for the design of biomaterials which are applicable in antifouling, implant technology and immunosensors. Controlled immobilization of peptides onto artificial biointerfaces plays a key role in these technologies and it is of crucial importance to develop tools to examine interfacial properties of adsorbed peptides such as orientation, and secondary structure. In this study we used sum frequency generation (SFG) vibrational spectroscopy and near edge X-ray absorption fine structure (NEXAFS) spectroscopy to characterize the structure of α-helix and β-strand model peptides on self-assembled monolayers (SAMs). The formation of peptide monolayers was confirmed using X-ray photoelectron spectroscopy. The α-helix peptide is a 14-mer and the β-strand is a 15-mer of hydrophilic lysine (K) and hydrophobic leucine (L) residues with a hydrophobic periodicity of 3.5 and 2, respectively. Both peptides have the hydrophobic side-chains on one side and the hydrophilic on the other. The SAMs used as hydrophobic and hydrophilic model surfaces were prepared from alkane thiols on gold having either charged COOH or hydrophobic CH_3 units as terminal groups. For SFG studies we used the deuterated analog of the latter SAM. SFG spectra collected in the C-H region exhibit strong peaks near 2965 cm^{-1}, 2940 cm^{-1} and 2875 cm^{-1} related to ordered leucine side chains on both surface chemistries. The relative phase of these features revealed the orientation of the leucine side chains. On COOH a relative phase of 1.4 and 1.6 rad for α-helix and β-strand, respectively, showed that the leucine was oriented away from the surface while a phase of 0 rad for both peptides on CH_3 proved that the leucines are oriented towards the interface. Amide I peaks observed at 1656 cm^{-1} for the α-helix peptide confirm that the secondary structure is preserved on both SAMs. A strong linear dichroism related to the amide π^* orbital at 400.1 eV was observed in the nitrogen K-edge NEXAFS spectra for the β-strand peptides on both surfaces, suggesting that the peptides are oriented parallel to the surface with the side-chains normal to the interface. For the α-helix the dichroism of the amide π^* is weak, probably due to the broad distribution of amide bond orientations for this secondary structure.

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