Biomaterial Interfaces
Room: K - Session BI-TuM

Proteins and Cell Interactions at Interfaces II
Moderator: H.E. Canavan, University of New Mexico, S.L. McArthur, Swinburne University of Technology, Australia

8:00am **BI-TuM1 Molecular Simulation as a Surface Design Tool to Understand and Control Protein-Surface Interactions**, ***R.A. Latour***, Clemson University **INVITED**

Protein-surface interactions are of great importance in a wide variety of applications in biomedical engineering and biotechnology, including medical implants, biocatalysis, immobilized-enzyme bioreactors, biosensors, bioseparations, and bioanalytical systems. While this is well recognized, very little is understood regarding how to design surfaces to optimally control protein adsorption behavior. To address this limitation, we are working on the development of molecular simulation methods to accurately predict protein-surface interactions at the atomic level. We have found that this type of molecular system is sufficiently unique that molecular simulation methods cannot simply be borrowed from other applications; but rather, they must be critically evaluated and often modified to accurately represent adsorption behavior. In this talk, I will address four major areas that we have identified as being particularly important for the simulation of protein-surface interactions, and I will present our approaches to address each of these areas. These are (1) the general methods that are needed to properly simulate protein-surface interactions, (2) the suitability of a force field to represent protein-surface interactions, (3) the adequate treatment of solvation effects, and (4) the need for advanced sampling methods for large molecular systems. I will present an overview of our efforts to address each of these key areas. We are developing a hybrid force field program that enables multiple force fields to be used in a single simulation to represent different phases of a system (e.g., solid surface, solution, and the interphase between them), methods to enable pressure to be properly monitored and controlled in a simulation with constrained atoms, and how electrostatic effects should be represented for surfaces with high charge density when using periodic boundary conditions. We have generated a large experimental benchmark data set for peptide-surface interactions for use for force field evaluation, modification, and validation purposes along with simulation methods to calculate adsorption free energy for comparison with this data set. Regarding solvation effects, we have found that existing implicit solvation methods are completely unsatisfactory at this time and must be redeveloped before use in protein adsorption simulations. Finally, we are also developing advanced sampling methods for large molecular systems to efficiently overcome energy barriers that often cause simulations to become trapped in local low-energy states and prevent proper exploration of the relevant phase space of the molecular system.

8:40am **BI-TuM3 Surface-Induced Changes in the Structure of Beta-Helical Peptides**, ***K. Fears***, *J. Kulp III, D.Y. Petrovykh, T. Clark*, US Naval Research Laboratory

The stable structure of beta-helical peptides in solution provides a well-defined starting point for discerning the changes in secondary structure of peptides induced by surface adsorption. Understanding the adsorption of proteins on surfaces is of critical importance in medical- and bio-technology. The determination of the secondary and higher-order structure of adsorbed proteins, however, is challenging due to their inherent complexity. Peptides with simple secondary structures provide a good model for investigating the interactions between surfaces and the structural subunits of proteins. Beta-helical, rather than alpha-helical, peptides were selected for this study because of their stability and tendency to maintain a monomeric, unaggregated structure. Peptides were custom designed to switch between two different stable conformations as a function of solvent composition, as confirmed by nuclear magnetic resonance (NMR) and circular dichroism (CD) spectroscopies in solution. These peptides were adsorbed onto silica substrates from aqueous and organic solvents to determine their conformation post-adsorption. The secondary structure assignments of the adsorbed peptides were based on the comparison of their CD spectra to spectra of the same peptides in their known solution conformation. Our ability to measure the secondary structure of peptides that are adsorbed as monolayers on planar substrates suggests that CD spectroscopy can be used to gain insight about the adsorption behavior of individual subunits that provide support and/or functionality to proteins.

9:00am **BI-TuM4 Enantiospecific Adsorption of Serine Enantiomers on the Chiral Cu{531} Surface**, *T. Eralp, A. Shavorskiy,* University of Reading, UK, *D. Batchelor,* BESSY and Universität Würzburg, Germany, ***G. Held***, University of Reading, UK

The production of enantiopure chiral bio-relevant molecules is of significant importance for the development of new pharmaceuticals and the improvement of existing ones. In this context chiral surface systems, e.g. chiral molecules adsorbed on chiral single crystal surfaces, are of considerable interest as they are model systems for potential enentioselective heterogeneous catalysts or enantiomeric selection. In this study the adsorption properties of L- and D- Serine enantiomers on the intrinsically chiral Cu{531} surface were investigated. These aminoacids have four functional groups which can make bonds to the Cu{531} surface: OH, NH_2, and two oxygen atoms in the carboxyl group (-COOH). The geometry of the adsorption complex was characterised using XPS and NEXAFS. The bonding characteristics of the molecule strongly depend on the coverage. The main peak in the O1s XPS spectra, at BE 531.5eV, is assigned to be overlapping signal of the two oxygen atoms in the deprotonated carboxylate (COO) group forming bonds with Cu atoms. For the low coverages this peak has a shoulder at a lower BE (530.7 eV), as the coverage increases this shoulder disappears and a new peak appears at higher BE (532.8 eV). The low BE shoulder at low coverage is assigned to the OH group also forming a bond with the Cu surface. With increasing coverage the surface becomes more crowded and a less space-consuming configuration is assumed with a 'dangling' OH group, which is the origin of the high BE O1s peak.

In order to investigate the orientation of the amino acids within the surface NEXAFS spectra were recorded for different in-plane polarization angles and different coverages. These spectra show large enantiomeric differences between the orientation of D-serine and L-serine, which is also reflected in the LEED patterns, indicating different long-range ordered overlayers. These enantiomeric differences are much bigger than those previously observed for alanine on the same surface, which suggests that the size and the nature of the amino acid side group is the dominating factor for enantioselective behaviour on this surface.

9:20am **BI-TuM5 Investigation of Surface-Bound Protein Conformation/Orientation Using Time-of-Flight Secondary Ion Mass Spectrometry**, ***L.J. Gamble***, *F. Cheng, J. Brison, L. Árnadóttir, D.G. Castner*, University of Washington

The adsorption of protein to solid surfaces is typically accompanied by structural rearrangements as well as loss of bioactivity. These changes can be monitored by time-of-flight secondary ion mass spectrometry (ToF-SIMS) and the protein activity monitored by surface plasmon resonance (SPR). However, the ultra-high vacuum of the ToF-SIMS can alter the protein conformation. In this study ToF-SIMS was coupled with a variable temperature sample stage to monitor the conformational changes that occur when a surface-bound protein goes from a hydrated to a dehydrated state. Changes in bioactivity of the surface bound proteins were investigated using SPR. Initial ToF-SIMS and SPR experiments were conducted on a surface-bound protein system of histagged humanized anti-lysozyme variable fragment (HuLys Fv) coordinated on a Ni^{2+}-loaded nitrilotriacetic acid (NTA) surface. Continuing studies investigate fibrinogen (and fibrinogen fragments). Positive ToF-SIMS data from the protein surfaces were acquired with an ION-TOF TOF.SIMS 5-100 system (ION-TOF GmbH, Münster, Germany) . Applying principal component analysis (PCA) to the ToF-SIMS data, the spectral differences resulting from two surface coverages and various heat treatments were determined. The spectra are separated into three groups: high protein coverage samples, low coverage below -80ºC, and low coverage at -60ºC and above. Trends observed in the plot suggest both surface coverage and heat treatment affected the secondary ion spectra. At the temperature below about -80oC, the protein molecules are frozen into their hydrated conformation. As the temperature is raised changes expose hydrophobic amino acid residues. The antigen binding capacity of surface-bound HuLys Fv before and after dehydration was measured by SPR. At the low coverage, the antigen binding capacity on the dried protein film was roughly 50% lower than that on the fresh film. As comparison, high coverage dried samples lost ~20% binding capacity. The loss of HuLys Fv bioactivity on the dried protein film was attributed to an irreversible disruption of protein native conformation during the drying process. The high coverage samples exhibited less loss of bioactivity, consistent with the smaller conformational changes observed by PCA. The use of the sugar trehalose as a protein stabilizer is also investigated.

9:40am **BI-TuM6 Soft X-ray Spectromicroscopy of Protein Interactions with Model Biomaterials**, ***B.O. Leung***, *A.P. Hitchcock, J.L. Brash,* McMaster University, Canada, *A. Scholl, A. Doran*, Advanced Light Source

Upon implantation in biological tissue or first contact with blood, all materials are immediately coated with a layer of proteins. The details of this initial protein layer can have a very strong effect on biocompatibility [1]. Thus, characterization of the surfaces of biomaterials and their interaction with relevant proteins can help to determine and understand biocompatibility.

We use synchrotron based X-ray photoemission electron microscopy (X-PEEM) [2] and scanning transmission X-ray microscopy (STXM) [3] to study the spatial distribution of adsorbed proteins on chemically heterogeneous surfaces. Both techniques have a lateral spatial resolution below 40 nm, provide speciation and quantitation through spatially resolved near-edge X-ray absorption fine structure (NEXAFS) spectroscopy, and can be used to obtain quantitative maps of the adsorbed protein in relation to the topography and chemistry of the underlying substrate with high sensitivity [4-5]. X-PEEM probes the top 10 nm of the sample whereas STXM integrates over the full sample thickness (40-100 nm) and can be applied to fully hydrated samples.

Results from three recent studies will be presented. (1) spun-cast thin films of phase-segregated polystyrene blended with cross-linked polyethylene oxide (PEO) adsorb human serum albumin (HSA) more readily to the PEO -rich areas compared to plasma-deposited diglyme surfaces, which are known protein resistant materials. Due to incomplete phase segregation, PEO imparts some protein resistance to the PS region. (2) HSA adsorption to polystyrene/ poly(methyl methacrylate)-b-polyacrylic acid (PS/PMMA-b-PAA) thin films show strong modifications of the adsorption behavior relative to HSA adsorption to PS/PMMA surfaces due to strong and specific electrostatic interactions between the positively charged peptide and the negatively charged PMMA-b-PAA domains. (3) Protein adsorption to PS-PMMA spun-cast films has been examined by STXM in a fully hydrated wet cell. In the presence of water, HSA preferentially adsorbs to the discrete polar PMMA domains rather than the continuous PS domain, whereas the latter is favored under dried conditions [2]. [6]

1. D. G. Castner, and B. D. Ratner, *Surface Science* **2002**, *500*, 28 – 60.

2. L.Li, J. Brash, R. Cornelius and A. P. Hitchcock *J. Phys. Chem B* **2008,** *112*, 2150-58

3. H. Ade and A.P. Hitchcock, *Polymer* **2008,** *49,* 643-675.

4. B. O. Leung, A. P. Hitchcock et al. *Macromolecules* **2009**, *42*, 1679 – 1684.

5. B. O. Leung, A. P. Hitchcock et al. *Biomacromolecules* **2009**, in press

6. Research carried out with PEEM2 and STXM532 at the Advanced Light Source, which is supported by Basic Energy Sciences, DoE, USA. Research funded by NSERC.

10:40am **BI-TuM9 Probing Individual Side-Chains of Peptides at Interfaces Using Isotope Labeling with Sum Frequency Generation Spectroscopy**, ***T. Weidner***, *N.F. Breen, G.P. Drobny, D.G. Castner*, University of Washington

Controlled immobilization of peptides onto artificial biointerfaces plays a key role in antifouling, implant and immunosensor technologies and it is of crucial importance to develop tools to examine interfacial properties of adsorbed peptides. Sum frequency generation (SFG) spectroscopy can probe biomolecules at the solid-liquid interface. Isotope labeling can address specific protein regions but its potential in conjunction with SFG spectroscopy has remained mostly unexplored. We combine these techniques to characterize the structure of synthetic model peptides on surfaces in PBS buffer. The peptide used is a 14-mer of hydrophilic lysine (K) and hydrophobic leucine (L) residues with an α-helical secondary structure. These LK14 peptides have the hydrophobic side-chains on one side of the helix and the hydrophilic on the other. Deuteration of the isopropyl group of each of the leucines, totaling 8 samples, was used to probe individual leucine side chains of LK14 adsorbed onto a hydrophobic polystyrene surface. Side-chain orientations were determined using ratios of the asymmetric CD_3 stretching mode at 2221 cm^{-1} acquired with different polarization combinations. We found that the orientation of the leucine side chains in the surface-bound LK14 was remarkably different from the calculated solution structure. Leucines in the center of the peptide are more oriented towards the surface while those at the ends of the amino-acid sequence are more bent away, indicating the leucines in the center of the peptide chain play a dominant role for the binding of the peptide. Solid state NMR data acquired on polystyrene beads is in line with the determined orientation change upon binding. In addition, ^{15}N labeling was used to address the controversial assignment of a pronounced peak near 3300 cm^{-1} observed for a variety of proteins adsorbed onto surfaces. This spectral feature has been assigned to both N-H containing side chains and backbone-related amide A resonances before. ^{15}N labeling of the lysine side chains resulted in a 9 cm^{-1} red-shift of this peak in the spectrum of LK adsorbed onto a SiO_2 surface, showing the 3300 cm^{-1} feature is related to the terminal amine group on the lysine side chains.

11:00am **BI-TuM10 Surface-initiated Vapor Deposition Polymerization of Poly(gamma-benzyl L glutamate): Optimization and Mechanistic Study**, ***W. Zheng***, *C.W. Frank*, Stanford University

Surface-initiated vapor deposition polymerization (SI-VDP) is a highly effective approach to synthesize grafted polypeptides. In this study, we developed an SI-VDP system having pressure and temperature control to reduce vacuum restriction 1000 times with high grafting efficiency and, thus, were able to synthesize grafted poly(gamma-benzyl L-glutamate)(PBLG) film of 167nm thick under 0.75 mbar. More importantly, we quantitatively investigated mechanistic details of the SI-VDP process including monomer vaporization and reservoir polymerization in the monomer reservoir and monomer condensation and physisorbed and chemisorbed polymerization on the substrate surface. To study the major monomer reservoir processes, we monitored the amount of vaporized monomers and developed a VDP reaction profile (VDPRP) method. We found that the VDPRPs were mostly contributed by the reservoir processes. We also found that characteristic features of the VDPRPs were determined by the monomer heating temperature and proposed possible mechanisms for the feature evolution. To evaluate the major substrate surface processes, we developed a quantitative analysis method using FTIR on both as-deposited PBLGs and chemisorbed PBLGs. Consequently, we were able to propose possible SI-VDP mechanisms leading to the surface-grafted PBLGs that were expected to have either high packing density with mostly α-helix segments or low packing density with a significant amount of both random coil and α-helix segments.

11:20am **BI-TuM11 Adsorbed α-Helical Polypeptides: Molecular Organization, Structural Properties, and Interactions**, ***B. Atmaja***, Stanford University, *J.N. Cha,* University of California, San Diego, *C.W. Frank*, Stanford University

In this work, we have developed 11-mercaptoundecanoic acid (MUA)-polypeptide "bilayer" systems by adsorbing poly(diethylene glycol-L-lysine)-poly(L-lysine) (PEGLL-PLL) diblock copolypeptide molecules of various architectures onto MUA-functionalized gold substrates. Previously, we reported the self-assembly of PEGLL-PLL with nanoparticles that were functionalized with carboxylic acid (COOH) moieties to form a variety of supramolecular structures. In relation to this previous work, we have used the PEGLL-PLL/MUA bilayer as a model system for studying the interfacial phenomena that occur when the PEGLL-PLL molecules interact with the COOH moieties of nanoparticle ligands. Specifically, we have elucidated the nature of the interactions between the PEGLL-PLL and COOH moieties as well as the resulting polypeptide conformation and organization, using a combination of surface techniques—grazing-incidence infrared (IR) spectroscopy, ellipsometry, and contact angle. Because our PEGLL-PLL/MUA bilayer system can potentially be applied as a nonfouling surface, we have thoroughly characterized other film properties such as the packing and graft density of the polypeptide molecules as a function of the PEGLL-PLL architecture. A complete understanding of the film's molecular structure would then allow us to elucidate the relationship between the bilayer's nonfouling characteristics and its underlying structure in our future work. Using IR spectroscopy, the adsorption process is determined to occur primarily by means of electrostatic interaction between the protonated PLL residues (pKa ~ 10.6) and carboxylate moieties of the MUA SAM (pKa ~ 6) that is enhanced by H-bonding. The PLL block is thought to adopt a random-coil (extended) conformation, while the PEGLL block that is not interacting with the MUA molecules is found to adopt an α-helical conformation with an average tilt-angle of ~ 60°. The PEGLL-PLL molecules have also been deduced to form a heterogeneous film and adopt a liquid-like/disordered packing on the surface. The average contact angle of the polypeptide/MUA bilayer systems is ~ 40°, which implies that the diethylene glycol (EG2) side chains of the PEGLL residues may be oriented somewhat toward the surface normal. From ellipsometry measurements, it is found that PEGLL-PLL molecules with a longer α-helical block are associated with a lower graft density on the MUA surface compared to those with a shorter α-helical block. This observation may be attributed to the greater repulsion—steric and H-bonding effects—that is imposed by the EG2 side chains found on and projected area occupied by the longer PEGLL block.

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